Rapport de la Réunion de la Commission des normes sanitaires pour les animaux aquatiques de l'OMSA

Original : anglais (EN)

14 au 21 septembre 2022
Réunion en format hybride

Introduction et contribution des Membres

La Commission des normes sanitaires pour les animaux aquatiques de l'OMSA (ci-après désignée par « Commission des animaux aquatiques ») a souhaité remercier les Membres suivants de lui avoir adressé des commentaires écrits sur les projets de textes destinés au *Code sanitaire pour les animaux aquatiques* (ci-après désigné par « *Code aquatique* ») et au *Manuel des tests de diagnostic pour les animaux aquatiques* (ci-après désigné par « *Manuel aquatique* ») de l'OMSA et diffusés dans son rapport de février 2022 : l'Australie, le Canada, la Chine (Rép. populaire de), les États-Unis d'Amérique, le Japon, la Norvège, la Suisse, le Taipei chinois, la Thaïlande ainsi que les États membres de l'Union européenne (UE). La Commission a également souhaité remercier les nombreux experts du réseau scientifique de l'OMSA pour leurs précieux conseils et contributions.

La Commission des animaux aquatiques a pris en considération tous les commentaires reçus dès lors qu'ils étaient transmis dans les délais impartis et justifiés. En raison du grand nombre de commentaires reçus, la Commission n'a pas été en capacité de fournir des explications détaillées quant aux raisons motivant l'acceptation ou le rejet de chacune des propositions recueillies. Elle a réservé ses explications aux commentaires les plus importants. Elle n'a pas inclus d'explications justifiant les modifications d'ordre rédactionnel apportées au texte. La Commission a souhaité rappeler que les propositions de Membres visant à améliorer la clarté des textes n'ont pas toutes été acceptées ; elle a en effet considéré que dans les cas où le texte était clair tel que rédigé, elle n'en tiendrait pas compte. La Commission a procédé aux amendements des textes de la façon usuelle, c'est-à-dire par l'utilisation des fonctions « double souligné » et « barré » du logiciel de traitement de texte. Dans les annexes concernés, les amendements proposés lors de cette réunion sont mis en exergue par un surlignage en couleur afin d'être différenciés de ceux précédemment proposés.

À noter

La Commission des animaux aquatiques a informé les Membres que les rapports des groupes *ad hoc* ne seraient plus présentés dans les annexes de ses rapports. À la place, un lien hypertexte sera fourni afin de permettre au lecteur d'accéder aux pages dédiées à l'ensemble des rapports de groupes *ad hoc* du site internet de l'OMSA :

https://www.woah.org/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/

Statut des annexes

Les textes figurant en annexes 1 à 9 et 11 à 30 sont présentés afin que les Membres formulent des commentaires.

Procédure de soumission des commentaires

La Commission des animaux aquatiques encourage vivement les Membres et les Organisations internationales ayant signé un Accord de coopération avec l'OMSA à participer à l'élaboration des normes internationales de l'OMSA en soumettant des commentaires sur les annexes concernées du présent rapport.

Les commentaires doivent être transmis au format Word plutôt qu'au format pdf en raison des difficultés à incorporer le texte au format pdf dans les documents de travail de la Commission.

Les commentaires doivent être présentés dans les annexes concernées, et inclure toutes les propositions d'amendements du texte, dûment étayées par des arguments structurés ou par des références scientifiques publiées. Les propositions de suppression doivent être indiquées par des caractères ~~barrés~~ et celles d'ajouts par l'emploi du double soulignement. Les Membres ne doivent pas utiliser la fonction automatique « suivi des modifications » du logiciel de traitement de texte Word car les marques du suivi de correction disparaissent lors de l'intégration de leurs propositions aux documents de travail de la Commission.

Date limite de réception des commentaires

Les commentaires formulés sur les textes concernés du présent rapport devront être transmis au siège de l'OMSA avant le 6 janvier 2023 afin que la Commission des animaux aquatiques puisse les examiner lors de sa réunion de février 2023.

Organisation mondiale de la santé animale
Fondée en tant qu'OIE

Service des Normes
[ACC.Secretariat@woah.org]

12, rue de Prony
75017 Paris, France

T. +33 (0)1 44 15 18 88
F. +33 (0)1 42 67 09 87
woah@woah.org
www.woah.org

Destinataire des commentaires

L'ensemble des commentaires devra être adressé au Service des normes, dont l'adresse électronique est : AAC.Secretariat@woah.org

Date de la prochaine réunion

La Commission a indiqué que sa prochaine réunion se tiendrait du 15 au 22 février 2023.

Sommaire

Liste des annexes

1. Accueil

1.1. Directrice générale adjointe de l'OMSA pour le Service des Normes internationales et la Science

La docteure Montserrat Arroyo, Directrice générale adjointe de l'OMSA pour le Service des Normes internationales et Science, a accueilli les membres de la Commission des animaux aquatiques et les a remerciés pour leur contribution aux travaux de l'OMSA. La docteure Arroyo les a félicités pour leur ordre du jour ambitieux et a exprimé sa reconnaissance envers les institutions et gouvernements des Membres les employant.

La docteure Arroyo a informé la Commission du projet d'organiser la 90e Session générale en présentiel, l'objectif étant de recréer du lien après les dernières Sessions générales, tenues en visioconférence ou selon un format hybride. Elle a encouragé les membres de la Commission à présenter les faits marquants du rapport de réunion de septembre 2022 dans les webinaires régionaux car cela constituerait un excellent moyen de renforcer l'implication des Membres. Elle a également informé la Commission que le nouvel acronyme de l'organisation, OMSA, serait progressivement introduit dans le *Code aquatique* et le *Manuel aquatique*. La docteure Arroyo a présenté une synthèse des initiatives en cours pour la numérisation de l'OMSA, et notamment le développement de nouveaux outils numériques et la planification pour leur mise en place. La docteure Arroyo a informé la Commission de la création du nouveau Réseau de coordination de la recherche de l'OMSA. Elle a fait un point rapide sur la mise en œuvre de la Stratégie de l'OMSA pour la santé des animaux aquatiques, tout en précisant que les membres de la Commission recevraient, pendant la réunion, d'autres présentations sur les activités menées dans ce cadre. Les membres de la Commission des animaux aquatiques ont remercié la docteure Arroyo pour l'excellent soutien apporté par le Secrétariat de l'OMSA.

1.2. Directrice générale

La docteure Monique Éloit, Directrice générale de l'OMSA, a rencontré les membres de la Commission des animaux aquatique le 21 septembre et les a remerciés pour leur soutien et leur engagement dans la réalisation des objectifs de l'OMSA. La docteure Éloit a informé la Commission que la mise en œuvre de la Stratégie de l'OMSA pour la santé des animaux aquatiques avait permis l'émergence d'initiatives positives mais qu'il était nécessaire d'instaurer un équilibre entre les activités visant à soutenir la réalisation de l'Objectif 1. « Normes » et celles visant à soutenir les trois autres Objectifs, afin d'améliorer la santé et le bien-être des animaux aquatiques dans tous les domaines. Elle a informé la Commission que le soutien et le renforcement des actions régionales et des capacités seraient améliorés et auraient un impact croissant. La docteure Éloit a fait le point sur la révision en cours du Système scientifique de l'OMSA et a insisté sur le fait que ce dernier devait prendre en compte les pratiques actuelles les plus adéquates et qu'il devait être maniable et réactif. La docteure Éloit a discuté du rôle de l'OMSA dans la prévention des maladies, et plus spécifiquement chez la faune sauvage. À ce titre, elle a présenté certains des travaux en cours de l'OMSA dans le Cadre de la santé de la faune sauvage. La Commission des animaux aquatiques a remercié la docteure Éloit pour ces informations.

2. Adoption de l'ordre du jour

Le projet d'ordre du jour a été adopté par la Commission. L'ordre du jour et la liste des participants sont présentés respectivement en annexe 1 et 2.

3. Coopération avec la Commission des normes sanitaires pour les animaux terrestres

Le bureau (comprenant un président et deux vice-présidents) de la Commission des normes sanitaires pour les animaux terrestres a rencontré celui de la Commission des normes sanitaires pour les animaux aquatiques lors d'une courte réunion organisée le 19 septembre 2022 et présidée par la Directrice générale adjointe de l'OMSA pour le Service des Normes internationales et Science. L'objectif de cette réunion était le partage d'informations et la garantie d'une approche harmonisée dans le cadre des révisions des chapitres horizontaux, le cas échant. Les deux Commissions se sont engagées à réunir les bureaux au moins une fois par an afin d'assurer une meilleure coordination. Les bureaux ont discuté de questions d'intérêt commun en lien avec le *Code Aquatique* et le *Code terrestre* et notamment de :

- L'approche retenue par les deux Commissions pour l'élaboration de leur plan de travail respectif et le choix des critères pour l'établissement des priorités dans les sujets à traiter ;

- L'approche qui sera retenue pour la révision de l'emploi de définitions du Glossaire (« Autorité compétente », « Autorité vétérinaire », « Services vétérinaires », « Services chargés de la santé des animaux aquatiques ») dans les Codes, rendue nécessaire à la suite de l'adoption de la version révisée de ces définitions en mai 2022. Les bureaux sont convenus de coordonner la révision de l'utilisation de ces termes afin de garantir une approche harmonisée. Leurs propositions seront communiquées aux Membres pour avis en février 2023 (voir point 6.1.) ;

- La proposition de nouveaux travaux sur la certification électronique. Les bureaux sont convenus d'ajouter la révision du chapitre 5.2. du *Code aquatique* et du *Code terrestre* à leur plan de travail respectif (voir point 6.3.) ;

- Les progrès de chaque Commission dans la réalisation des travaux de révision respectifs de la Section 4. Les bureaux sont convenus de se tenir mutuellement informés de l'avancement des travaux en question ;

- Les travaux de révision des chapitres 5.4. à 5.7. du *Code terrestre*. Le bureau de la Commission du Code est convenu de transmettre les termes de références à la Commission des animaux aquatiques et de poursuivre le partage de documents de travail au fur et à mesure de leur élaboration ;

- La révision du chapitre 6.10. « Usage responsable et prudent des agents antimicrobiens en médecine vétérinaire » du *Code terrestre*. Le bureau de la Commission du Code est convenu de communiquer le rapport du groupe de travail sur la résistance aux agents antimicrobiens et le projet de chapitre ainsi que de transmettre les documents de travail modifiés au regard des commentaires reçus.

4. Plan de travail et priorités

Des commentaires ont été formulés par l'Australie, le Canada, la Norvège et l'UE.

La Commission a examiné les commentaires qui lui ont été adressés.

La Commission a indiqué être en accord avec le commentaire selon lequel les Membres devaient être tenus informés de la disponibilité de dispositifs permettant de faciliter la génération de données aux fins d'établissement des couples temps/températures nécessaires à l'inactivation des agents pathogènes pour l'obtention de marchandises dénuées de risques. La Commission a indiqué que l'OMSA bénéficiait de deux dispositifs qui permettraient d'identifier les besoins et d'encourager la recherche, à savoir le nouveau Programme de coordination de la recherche et le nouveau réseau de Laboratoires de référence de l'OMSA, qui sont en cours de développement dans le cadre de la Stratégie pour la santé des animaux aquatiques, afin d'encourager la recherche dans ce domaine. La Commission a également amendé le modèle de rapport annuel des Centres de référence afin que soient systématiquement identifiés les domaines dans lesquels la recherche est insuffisante. Elle communiquera ces informations à des fins de traitement dans le cadre du Programme de coordination de la recherche de l'OMSA. Cet amendement devrait faciliter l'alimentation ces deux nouveaux dispositifs. La Commission a indiqué qu'une consultation avait été lancée afin d'actualiser le document intitulé « Safe commodity assessments for OIE listed aquatic animal diseases » publié en 2016. Le résultat de la consultation sera examiné par la Commission lors de sa réunion en février 2023 et utilisé pour mettre à jour les articles X.X.3. concernés du *Code aquatique*.

La Commission a indiqué être en accord avec le commentaire selon lequel toutes les propositions d'amendements du chapitres 4.2. « Zonage et compartimentation » devront également être examinés en lien avec le chapitre 4.3. « Application de la compartimentation ». La Commission a souhaité rappeler aux Membres que les amendements apportés au chapitre 4.2. ne concerneront que le zonage et que l'information relative à la compartimentation figurant actuellement dans ce chapitre sera examiné après l'adoption des amendements au chapitre 4.3. « Application de la compartimentation ». La Commission poursuivra ses travaux de mise à jour du Titre 4 conformément au plan de travail, leur avancement étant conditionné à la disponibilité des ressources.

La Commission a souhaité remercier un Membre pour l'appui offert dans l'élaboration d'un nouveau chapitre relatif aux échanges de matériel génétique. Elle a indiqué que ce sujet sera plus amplement discuté lors de l'élaboration du nouveau chapitre.

La Commission a examiné les projets d'élaboration de chapitre 5.X. « Échanges commerciaux d'animaux aquatiques ornementaux », de chapitre 5.Y. « Échanges commerciaux de matériel génétique » ainsi que d'amendement du chapitre 4.3. « Application de la compartimentation ». La Commission a ajouté ces chapitres à son plan de travail, discuté d'une proposition de plan pour chacun des chapitres afin d'établir le rythme de progression, les étapes de réalisation jusqu'à l'achèvement, et les délais de transmission aux Membres pour qu'ils formulent leurs commentaires.

La Commission a souhaité remercier un Membre qui a communiqué des éléments de preuve scientifiques sur la sensibilité d'espèces au virus de la virémie printanière de la carpe (voir point 5.5.). S'agissant de maladies ayant fait l'objet d'évaluations antérieures, la Commission a rappelé que lorsque des nouveaux éléments de preuve sont rendus disponibles, les évaluations de la sensibilité de nouvelles espèces ou les réévaluations de la sensibilité d'espèces sensibles connues doivent être à réalisées et sont ainsi ajoutées au plan de travail. La Commission a encouragé les Membres à lui transmettre tout nouvel élément de preuve à caractère scientifique sur la sensibilité des espèces aux fins des évaluations.

La Commission s'est entretenue avec le Service des Statuts de l'OMSA sur le processus d'auto-déclaration de statut indemne de maladie et sur l'amendement de la procédure actuelle pour la publication de l'auto-déclaration du statut sanitaire au regard des maladies animales afin qu'elle soit en ligne avec la version nouvellement adoptée du chapitre 1.4.

« Surveillance des maladies des animaux aquatiques ». La Commission est convenue d'ajouter un point dans son plan de travail sur le développement d'orientations additionnelles, qui prendraient la forme d'un modèle d'auto-déclaration afin d'accompagner les Membres lors de la soumission de leurs-auto-déclarations.

La Commission a examiné le statut d'avancement des sujets figurant dans son plan de travail et a fixé les étapes prévisionnelles nécessaires à leur traitement.

La Commission a examiné l'établissement des ordres de priorité des nouveaux sujets de travaux, en tenant compte d'un certain nombre de critères, notamment l'amélioration attendue des normes et leur impact, les bénéfices retirés par les Membres, les commentaires des Membres, la pertinence de ces sujets au regard des actions menées dans le cadre de la Stratégie de l'OMSA pour la santé des animaux aquatiques, les commentaires des services du Siège de l'OMSA et l'état d'avancement des travaux en cours figurant dans le plan de travail.

La Commission a indiqué qu'elle anticipait que l'avancement des travaux du plan de travail qui étaient conditionnés par l'établissement de groupes *ad hoc* progresserait comme prévu en 2022. La liste des groupes *ad hoc* existants ou à établir en 2022 est disponible sur le site internet de l'OMSA.

La version actualisée du plan de travail est présentée aux Membres en annexe 3 afin qu'ils formulent leurs commentaires.

Code sanitaire pour les animaux aquatiques de l'OMSA

5. Textes soumis aux Membres pour avis

5.1. Chapitre 1.3. « Maladies listées par l'OIE » – Inclusion de l'infection par des *Megalocytivirus*

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a informé les Membres que d'autres *Megalocytivirus* pouvaient également causer des maladies chez les poissons, bien qu'ils ne soient pas inclus dans le champ d'application du chapitre 2.3.7. « Infection par l'iridovirus de la daurade japonaise » du *Manuel aquatique*. La Commission a indiqué que l'inscription dans la Liste du virus de la nécrose infectieuse rénale et splénique (ISKNV), de l'iridovirus du corps rougeâtre du turbot (TRBIV) ou d'autres mégalocytivirus était conditionnée par la réalisation préalable d'une évaluation au moyen des critères d'inclusion dans la Liste des maladies du chapitre 1.2. du *Code aquatique*. En cas de satisfaction à ces critères, leur inclusion dans la Liste des maladies serait proposée à l'Assemblée mondiale des Délégués de l'OMSA.

Le groupe *ad hoc* sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE s'est réuni en avril 2022 afin de poursuivre ses travaux sur la sensibilité des espèces au moyen des critères figurant au chapitre 1.5. « Critères d'inclusion dans la liste des espèces sensibles à une infection par un agent pathogène spécifique ». Lors de cette réunion, le groupe ad hoc a conduit des travaux préliminaires d'évaluations de la sensibilité des espèces de poissons à l'infection par l'iridovirus de la daurade japonaise et a présenté un rapport intermédiaire à la Commission pour examen.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partie B, point 3.1.2.3, page 13).

Septembre 2022

La Commission a examiné le rapport du groupe *ad hoc* sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE concernant l'infection par l'iridovirus de la daurade japonaise. La Commission a également noté que la distinction des espèces sensibles à l'infection par l'iridovirus de la daurade japonaise nécessitait la réalisation d'analyses de la séquence de l'acide nucléique et/ou l'établissement d'arbres phylogénétiques afin de déterminer si l'agent pathogène appartenait au génogroupe RSIV ou à un autre génogroupe. Par conséquent, les éléments de preuves permettant au groupe ad hoc d'identifier les espèces sensibles à l'échelle du génotype faisaient parfois défaut. Au regard de ces éléments, le groupe ad hoc a achevé ses travaux préliminaires d'évaluation de la sensibilité des espèces de poissons à l'infection par l'iridovirus de la daurade japonaise, à l'infection par le virus infectieux de la nécrose rénale et splénique infectieuse (ISKNV) et à l'infection par l'iridovirus du corps rougeâtre du turbot (TRBIV) et a recommandé à la Commission, dans son rapport intermédiaire, d'envisager l'inclusion des virus appartenant à l'échelon taxonomique des *Megalocytivirus*, notamment le virus de la nécrose infectieuse rénale et splénique, l'iridovirus de la daurade japonaise et l'iridovirus du corps rougeâtre du turbot (mais pas le virus de la maladie de perte d'écailles (SDDV), génétiquement plus éloigné)

La Commission est convenue qu'en raison de la complexité liée à l'existence de différents génogroupes au sein du genre *Megalocytivirus*, il était nécessaire de procéder à des évaluations pour chacun d'entre eux au moyen des critères figurant dans l'article 1.2.2. du chapitre 1.2. « Critères d'inclusion des maladies des animaux aquatiques dans la liste de l'OIE ». La Commission a décidé d'évaluer les espèces de virus de la nécrose infectieuse rénale et splénique (ISKNV), et notamment ses trois génogroupes : RSIV, ISKNV et TRBIV. Elle a conclu que le génogroupe RSIV (actuellement listé dans le *Code aquatique*) ainsi que les deux génogroupes ISKNV et TRBIV satisfaisaient aux critères 1, 2, 3 et 4b.

La Commission a noté que les virus des trois génogroupes (RSIV, ISKNV et TRBIV) présentaient des similitudes en matière d'espèces sensibles, d'épidémiologie et de méthodes de diagnostic. À ce titre, la Commission a estimé que la maladie devait être listée sous la désignation « Infection par le virus de la nécrose infectieuse rénale et splénique (ISKNV) ». L'infection par le virus de la nécrose infectieuse rénale et splénique inclura les trois génogroupes de l'espèce ISKNV (c'est-à-dire ISKNV, RSIV et TRBIV) mais exclura l'autre espèce reconnue de *Megalocytivirus,* le SDDV.

L'évaluation de l'infection par le virus de la nécrose infectieuse rénale et splénique en vue de son inclusion dans la Liste des maladies de l'OMSA est présentée aux Membres en annexe 5 afin qu'ils formulent leurs commentaires.

La version révisée de l'article 1.3.1. du chapitre 1.3. « Maladies listées par l'OIE » est présentée aux Membres en annexe 4 afin qu'ils formulent leurs commentaires.

5.2. Article 9.3.1. du chapitre 9.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) »

Des commentaires ont été formulés par la Chine (Rép. populaire de), la Norvège, la Suisse et l'UE.

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a décidé d'amender l'article 9.3.1., afin d'en assurer la cohérence avec le chapitre 1.3. « Maladies listées par l'OIE ».

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février (partie B : point 2.1.2., page 6).

Réunion de septembre 2022

La Commission a accepté la proposition de modifier la description taxonomique figurant dans l'article 9.3.1. et, conformément à la convention utilisée dans le *Code aquatique* et le *Manuel aquatique*, elle a ajouté l'échelon de la famille et amendé l'ordre en « Rickettsiales » afin que le texte soit en ligne avec la classification taxonomique reconnue. La Commission a également amendé la section 1. du chapitre 2.2.3. du *Manuel aquatique* à des fins d'harmonisation (voir le point 7.1.4).

La version révisée de l'article 9.3.1. du chapitre 9.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) » est présentée aux Membres en annexe 6 afin qu'ils formulent leurs commentaires.

5.3. Articles 9.4.1. et 9.4.2. du chapitre 9.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse »

Des commentaires ont été formulés par la Norvège, la Suisse et l'UE.

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques est convenue d'amender le texte de l'article 9.4.1. du chapitre 9.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse » afin de prendre en compte la mise à jour de la classification taxonomique du virus de la nécrose hypodermique et hématopoïétique infectieuse ainsi que d'en assurer l'alignement sur le texte des autres chapitres spécifiques aux maladies.

Dans l'article 9.4.2., la Commission est convenue d'amender la liste des espèces sensibles en ligne avec la convention utilisée dans l'article X.X.2. du *Code aquatique*, consistant à lister le nom vernaculaire des espèces sensibles par ordre alphabétique. La Commission a également amendé la section 2.2.2. du chapitre 2.2.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse » du *Manuel aquatique* à des fins d'harmonisation.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 report (partie B : point 2.1.3., page 6).

Réunion de septembre 2022

La Commission a examiné les commentaires qui lui ont été adressés. Constatant que les Membres étaient favorables aux propositions de modifications, elle n'a pas proposé d'amendements supplémentaires.

La version révisée des articles 9.4.1. et 9.4.2. du chapitre 9.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse » est présentée aux Membres en annexe 7 afin qu'ils formulent leurs commentaires.

5.4. Article 9.5.2 du chapitre 9.5. « Infection par le virus de la myonécrose infectieuse »

Contexte

Dans un effort constant de garantir l'harmonisation entre le *Code aquatique* et le *Manuel aquatique*, l'application progressive du modèle de chapitre spécifique aux maladies du *Manuel aquatique* s'accompagne également, si nécessaire, de la mise à jour des articles correspondants dans les chapitres spécifiques aux maladies du *Code aquatique.*

Réunion de septembre 2022

Dans l'article 9.5.2., la Commission est convenue d'amender la liste des espèces sensibles en ligne avec la convention utilisée dans l'article X.X.2. du *Code aquatique*, et qui consiste à lister le nom vernaculaire des espèces sensibles par ordre alphabétique. La Commission a également amendé la section 2.2.2. du chapitre 2.2.5. « Infection par le virus de la myonécrose infectieuse » du *Manuel aquatique* à des fins d'harmonisation (voir le point 7.1.6.).

La version révisée de l'article 9.5.2. du chapitre 9.5. « Infection par le virus de la myonécrose infectieuse » est présentée aux Membres en annexe 8 afin qu'ils formulent leurs commentaires.

5.5. Article 10.9.2. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe »

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques est convenue qu'en cas de disponibilité de nouveaux éléments de preuves sur la sensibilité des espèces d'animaux aquatiques aux maladies listées par l'OIE, es évaluations de la sensibilité de nouvelles espèces ou les réévaluations de la sensibilité d'espèces sensibles connues devraient être réalisées. La Commission a ajouté un point dédié et pérenne dans son plan de travail afin de traiter ce sujet. La Commission a encouragé les Membres à lui transmettre tout nouvel élément de preuve sur la sensibilité des espèces aux fins des évaluations.

Réunion de septembre 2022

En réponse à un Membre fournissant des éléments de preuve de la sensibilité d'une nouvelle espèce, la Commission a demandé que le groupe ad hoc sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE évalue la sensibilité de *Percocypris pingi* à l'infection par le virus de la virémie printanière de la carpe.

La Groupe *ad hoc* a repris et appliqué les critères présentés dans son rapport de novembre 2017 sur la sensibilité des espèces de poissons à l'infection par le virus de la virémie printanière de la carpe afin d'évaluer la sensibilité de *Percocypris pingi*.

La Commission a examiné l'évaluation du groupe *ad hoc* et est convenue d'inclure *Percocypris pingi* dans la liste des espèces sensibles de l'article 10.9.2.

L'évaluation de la sensibilité de *Percocypris pingi* réalisée par le groupe *ad hoc* est présentée aux membres en annexe 10 à titre informatif.

La version révisée de l'article 10.9.2. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe » est présentée aux Membres en annexe 9 afin qu'ils formulent leurs commentaires.

5.6. Nouveau chapitre 10.X. « Infection par le virus du tilapia lacustre »

Contexte

Suite à l'adoption de l'inclusion de l'infection par le virus du tilapia lacustre (TiLV) dans l'article 1.3.1. du chapitre 1.3. « Maladies listées par l'OIE », en mai 2022, la Commission des animaux aquatiques est convenue d'élaborer un nouveau projet de chapitre relatif à l'infection par le TiLV, selon le format de chapitre (structure des articles) figurant dans le Titre 10 du *Code aquatique*.

Réunion de septembre 2022

La Commission a révisé le projet de chapitre 10.X. « Infection par le virus du tilapia lacustre », élaboré par l'un de ses membres.

La Commission a souhaité rappeler aux Membres que la mention « À l'étude » figurant dans le projet de chapitre 10.X. « Infection par le virus du tilapia lacustre » signifie que « le texte n'a pas été adopté par l'Assemblée mondiale des Délégués auprès de l'OMSA et qu'il ne fait donc pas partie intégrante du *Code aquatique* », conformément au point B.2. du guide de l'utilisateur.

La Commission a décidé que la liste des espèces sensibles listées dans l'article 10.X.2. serait considérée comme étant « à l'étude » dans l'attente des résultats de l'évaluation réalisée au moyen des critères du chapitre 1.5. « Critères d'inclusion dans la liste des espèces sensibles à une infection par un agent pathogène spécifique ». La Commission a également décidé de placer les produits issus d'animaux aquatiques listés aux points 1 et 2 de l'article 10.X.3. et au point 1a. de l'article 10.X.14. comme étant « à l'étude », dans l'attente des résultats de l'évaluation au moyen des critères du chapitre 5.4. « Critères d'évaluation de la sécurité des marchandises issues d'animaux aquatiques ». La Commission est convenue que les procédés chimiques et physiques utilisés dans la production d'huile de poisson et de cuir de poisson étaient suffisants pour inactiver le virus du tilapia lacustre présent et que, par conséquent, les critères figurant au point 2. de l'article 5.4.1. étaient satisfaits. La Commission a donc décidé d'inclure ces produits issus d'animaux aquatiques dans l'article 10.X.3. plutôt que de les placer « à l'étude ».

La Commission est convenue que les périodes minimales pour les conditions élémentaires de sécurité biologique et pour la surveillance ciblée présentées dans le chapitre 1.4. « Surveillance des maladies des animaux aquatiques » seraient appliquées à l'infection par le virus du tilapia lacustre tant que l'évaluation de périodes minimales spécifiques n'aurait pas été achevée. La Commission a indiqué qu'elle avait sollicité les conseils d'un expert sur l'évaluation des périodes minimales pour l'ensemble des maladies listées, y compris l'infection par le virus du tilapia lacustre. Lorsque l'avis aura été émis et examiné par le Commission, des modifications pourront être proposées dans les chapitres spécifiques aux maladies, s'il y a lieu.

La version du nouveau projet de chapitre 10.X. « Infection par le virus du tilapia lacustre » est présentée aux Membres en annexe 11 afin qu'ils formulent leurs commentaires.

5.7. Article 11.2.2. du chapitre 11.2. « Infection à *Bonamia exitiosa* » et article 11.3.2. du chapitre 11.3. « Infection à *Bonamia ostreae* »

Contexte

Dans le rapport de juin 2022 du groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE, consacré à la sensibilité des espèces à l'infection à *Bonamia exitiosa* et à l'infection à *Bonamia ostreae*, il a été indiqué que *Magallana gigas* était le nom scientifique accepté dans la base WoRMS (the World Registry of Marine Species) pour désigner l'huitre creuse du Pacifique. Précédemment, le groupe *ad hoc* avait décidé de conserver le nom scientifique *Crassostrea gigas*, considérant que les éléments de preuve n'étaient pas suffisamment robustes pour étayer la proposition de modification de la classification taxonomique de cette espèce. Lors de la réunion du groupe ad hoc de mai-juin 2022 en vue d'évaluer la sensibilité des espèces à l'infection à *Marteilia refringens*, de nouvelles données et des publications revues en comité de lecture sur le nouveau nom *Magallana gigas* ont été examinées. Le groupe *ad hoc* a recommandé à la Commission des animaux aquatiques de modifier le nom scientifique de l'huitre creuse du Pacifique.

Le rapport du groupe *ad hoc* de juin 2022 est disponible sur le site internet de l'OMSA.

Réunion de septembre 2022

La Commission des animaux aquatiques a examiné le rapport du groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE pour l'infection à *Marteilia refringens*. Elle a noté que les recommandations du groupe *ad hoc* sur la nouvelle désignation de l'huitre creuse du Pacifique avaient des implications sur le texte des articles 11.2.2. du chapitre 11.2. « Infection à *Bonamia exitiosa* » et l'article 11.3.2. du chapitre 11.3. « Infection à *Bonamia ostreae* ».

La Commission est convenue d'amender, dans l'ensemble des chapitres du *Code aquatique* et du *Manuel aquatique* où ils sont cités, le nom scientifique de « *Crassotrea ariakensis* » en « *Magallana* (syn. *Crassotrea*) *ariakensis* » et celui de l'huitre creuse du Pacifique en « *Magallana* (syn. *Crassostrea*) *gigas* ». La Commission a décidé de procéder à ces amendements de façon progressive et d'amender les articles 11.2.2. et 11.3.2. lorsque le groupe *ad hoc* aurait réalisé l'évaluation de la sensibilité de ces espèces. Les amendements apportés aux autres articles 11.X.2. seront apportés au fur et à mesure des progrès accomplis par le groupe *ad hoc* dans ses travaux d'évaluation de la sensibilité aux autres maladies des mollusques. Pour les modifications correspondantes dans les sections 2.2.1. et 2.2.2. du chapitre 2.4.2. « Infection à *Bonamia exitiosa* » et du chapitre 2.4.3. « Infection à *Bonamia ostreae* » du *Manuel aquatique*, il faut se référer au point 7.3.1.

La version révisée de l'article 11.2.2. du chapitre 11.2. « Infection à *Bonamia exitiosa* » est présentée aux Membres en Annexe 12 afin qu'ils formulent leurs commentaires.

La version révisée de l'article 11.3.2. du chapitre 11.3. « Infection à *Bonamia ostrae* » est présentée aux Membres en Annexe 13 afin qu'ils formulent leurs commentaires.

5.8. Articles 11.4.1. et 11.4.2. du chapitre 11.4. « Infection à *Marteilia refringens* »

Contexte

Le groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE s'est réuni de novembre à décembre 2021 puis de mai à juin 2022 afin de poursuivre ses travaux d'évaluation au moyen des critères figurant au chapitre 1.5. « Critères d'inclusion dans la liste des espèces sensibles à une infection par un agent pathogène spécifique ». Lors de ces réunions, le groupe *ad hoc* a réalisé des évaluations de la sensibilité d'espèces de mollusques à l'infection à *Marteilia refringens*.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partie B : point 4.1., page 17).

Réunion de septembre 2022

La Commission des animaux aquatiques a examiné le rapport du groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE et félicité ses membres pour le travail exhaustif accompli.

La Commission a amendé l'article 11.4.1. afin qu'il soit en adéquation avec l'approche adoptée pour la révision de cet article dans les autres chapitres spécifiques aux maladies de mollusques.

La Commission est convenue d'amender la liste des espèces sensibles figurant dans l'article 11.4.2. en ligne avec les recommandations suivantes du groupe *ad hoc* :

- la sensibilité des trois espèces actuellement listées dans l'article 11.4.2., la moule commune (*Mytilus edulis*), l'huitre plate européenne (*Ostrea edulis*) et la moule méditerranéenne (*Mytilus galloprovincialis*), a été évaluée au moyen des critères d'inclusion dans la liste des espèces sensibles à l'infection à *Marteilia refringens*. Il est ainsi proposé qu'elles demeurent dans l'article 11.4.2.

- la sensibilité de cinq nouvelles espèces, l'huitre naine (*Ostrea stentina*), le couteau d'Europe (*Solen marginatus*), *Xenostrobus securis* et la petite praire (*Chamelea gallina*) a été évaluée au moyen des critères d'inclusion dans la liste des espèces sensibles à l'infection à *Marteilia refringens*. Il est ainsi proposé qu'elles soient incluses dans l'article 11.4.2.

- la sensibilité des trois espèces actuellement listées dans l'article 11.4.2., *Ostrea angasi*, *Ostrea puelchana* et l'huitre plate chilienne (*Ostrea chilensis*), a été évaluée au moyen des critères d'inclusion dans la liste des espèces sensibles à l'infection à *Marteilia refringens*. Il est ainsi proposé qu'elles soient supprimées de l'article 11.4.2.

La Commission n'a pas accepté la recommandation du groupe *ad hoc* d'inclure une espèce de copépode (*Paracartia grani*) dans l'article 11.4.2. bien qu'il satisfasse aux critères d'inclusion dans la liste des espèces sensibles à *M. refringens*. La Commission a en effet estimé qu'il n'était pas pertinent d'inclure cette espèce dans la liste aux fins des échanges de mollusques ou de produits issus de mollusques. La Commission a toutefois estimé que le copépode *Paracartia grani* devait être inclus dans la section 2.2.1. du chapitre 2.4.4. « Infection à *Marteilia refringens* » du *Manuel aquatique* pour s'assurer que les Membres soient informés que cette espèce est sensible et qu'à ce titre, sa prise en compte, lors de leurs contrôles, sous certaines conditions, pour prévenir sa propagation, peut être pertinente.

Les sections correspondantes du chapitre 2.4.4. « Infection à *Marteilia refringens* » du Manuel aquatique ont également été amendées en ligne avec les recommandations du groupe *ad hoc* (voir le point 7.3.2.)

La Commission a encouragé les Membres à se référer au rapport de juin 2022 du groupe *ad hoc*, disponible sur le site internet de l'OMSA, pour disposer du détail des évaluations qu'il a réalisées.

La version révisée des articles 11.4.1. et 11.4.2. du chapitre 11.4. « Infection à *Marteilia refringens* » est présentée aux Membres en Annexe 14 afin qu'ils formulent leurs commentaires.

5.9. Modèles d'articles 11.X.9. à 11.X.14. destinés aux chapitres spécifiques aux maladies des mollusques

Contexte

Lors de sa réunion en février 2018, la Commission des animaux aquatiques était convenue d'appliquer les modèles d'articles X.X.8., X.X.9., X.X.10. et X.X.11 à l'ensemble des chapitres spécifiques aux maladies des Titres 8, 9 et 10 du *Code aquatique.* Plus spécifiquement, la Commission avait décidé qu'elle introduirait les modifications figurant dans les modèles d'articles du Titre 11 des chapitres spécifiques aux maladies des mollusques au moment où elle procéderait aux amendements résultant des travaux entrepris par le groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2018 (point 1.11., page 13).

Réunion de septembre 2022

La Commission a noté que les amendements qui ont été apportés aux Titres 8, 9 et 10 n'avaient pas été systématiquement apportés dans les chapitres du Titre 11 comme cela avait été décidé antérieurement.

La Commission a amendé le texte figurant dans les articles 11.X.9. à 11.X.5., 11.X.6. ou 11.X.7. (selon le cas) et 11.X.8. afin qu'il soit en ligne avec celui figurant dans les articles X.X..5 à X.X.8. nouvellement adoptés. La Commission est convenue que le texte serait amendé dans les chapitres spécifiques aux maladies dès lors que les amendements proposés pour les articles 11.X.9. à 11.X.14. auraient été adoptés.

La Commission a indiqué que le nouvel article 11.X.13. avait été introduit afin que la structure du chapitre soit alignée sur celle des autres chapitres spécifiques aux maladies du *Code aquatique*, qui ont été adoptés en 2018. La Commission a également indiqué que l'article 11.X.4. comportait des informations spécifiques sur les marchandises ayant fait l'objet d'une évaluation, satisfaisant aux critères de l'article 5.4.2. et publiées dans l'édition de 2022 du *Code aquatique*. Les évaluations de la sécurité de ces marchandises sont disponibles dans le document intitulé « Safe commodities assessments for OIE listed aquatic animal diseases », publié sur le site internet de l'OMSA. La Commission a informé les Membres que l'information spécifique aux maladies n'avait pas été modifiée. Par conséquent, cette modification est présentée au point 1 du modèle d'article, qui présente l'information spécifique aux maladies entre […]. Les informations pertinentes figurant dans la version actuelle des chapitres spécifiques aux maladies sont incluses dans le tableau ci-après afin que les membres puissent en prendre connaissance. Elles seront incluses dans les chapitres spécifiques aux maladies au moment de l'adoption et de la publication.

Marchandises qui satisfont à l'article 5.4.2. « Critères d'évaluation de la sécurité des produits issus d'animaux aquatiques importés (ou en transit), destinés à la vente au détail pour la consommation humaine indépendamment du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de la maladie »

Chapitre	Agent pathogène	Marchandises
11.1.	Herpèsvirus de l'ormeau	a. chair d'ormeau éviscéré et décoquillé (à l'état réfrigéré ou congelé)
11.2.	*Bonamia exitiosa*	a. chair d'huitre à l'état réfrigéré et b. huitres en demi-coquille à l'état réfrigéré
11.3.	*Bonamia ostreae*	a. chair d'huitre à l'état réfrigéré et b. huitres en demi-coquille à l'état réfrigéré
11.4.	*Marteilia refringens*	a. chair de mollusque (à l'état réfrigéré ou congelé) et b. huitres en demi-coquille (à l'état réfrigéré ou congelé)
11.5.	*Perkinsus marinus*	a. chair de mollusque (à l'état réfrigéré ou congelé) et

		b. huitres en demi-coquille (à l'état réfrigéré ou congelé)
11.6	*Perkinsus olseni*	a. chair de mollusque (à l'état réfrigéré ou congelé) et b. mollusques en demi-coquille (à l'état réfrigéré ou congelé)
11.7.	*Xenohaliotis californiensis*	a. chair d'ormeau éviscéré et décoquillé (à l'état réfrigéré ou congelé)

La version révisée des modèles d'articles 11.X.9. à 11.X.14. est présentée aux Membres en annexe 15 afin qu'ils formulent des commentaires.

5.10. Questionnaire destiné aux Membres sur la révision du chapitre 4.3. « Application de la compartimentation »

Contexte

Lors de sa réunion en février 2022, la Commission a défini la révision du chapitre 4.3. « Application de la compartimentation » comme étant sa priorité première dans le cadre de la révision progressive du Titre 4 du *Code aquatique*. La Commission a choisi de modifier le chapitre 4.3. afin qu'il traite uniquement de la compartimentation, que les orientations y figurant et destinées aux Membres soient améliorées et qu'il soit en ligne avec les chapitres nouveaux ou révisés tels que le chapitre 4.1. « Sécurité biologique dans les établissements d'aquaculture ». La Commission a noté que la version révisée du chapitre 1.4. et les modèles d'articles X.X.4. à X.X.8. sur la déclaration du statut indemne destinés aux chapitres spécifiques aux maladies incluaient des références spécifiques aux exigences pour la démonstration et le maintien du statut indemne à l'échelle du compartiment. La Commission est convenue que la révision du chapitre 4.3. « Application de la compartimentation » serait la prochaine étape appropriée.

Réunion de septembre 2022

La Commission a estimé que les retours d'expérience des Membres sur l'utilisation et l''application de la compartimentation constitueraient une source d'information utile dans le cadre de la révision du chapitre 4.3. Par conséquent, la Commission a élaboré un court questionnaire (cinq questions) et a souhaité inviter les Membres à y répondre. Les réponses des Membres seront examinées lors de la réunion de la Commission en février 2023, qui poursuit ses travaux de révision du chapitre 4.3. afin que ce dernier réponde au mieux aux besoins des Membres.

Le questionnaire à visée participative est présenté aux Membres en Annexe 16 afin qu'ils formulent leurs commentaires.

6. Textes soumis aux Membres pour information

6.1. Définitions des termes « Autorité compétente », « Autorité vétérinaire » et « Services chargés de la santé des animaux aquatiques » figurant dans le glossaire

Contexte

En mai 2022, la version révisée des définitions des termes « Autorité compétente », « Autorité vétérinaire » et « Services chargés de la santé des animaux aquatiques » figurant dans le glossaire a été adoptée. La Commission des animaux aquatiques avait alors indiqué qu'une fois ces nouvelles versions adoptées, elle procéderait à un examen de leur utilisation dans l'ensemble du *Code aquatique*.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapports de septembre 2020 (point 4.5.3., page 9), septembre 2021 (point 5.1.2.2., page 7) et février 2022 (partie A : point 4.1.2.2., page 8).

Réunion de septembre 2022

Lors de la réunion des Bureaux de la Commission des animaux aquatiques et de la Commission du Code, il a été convenu que les deux Commissions coordonneraient leurs travaux respectifs de révision et d'amendement de l'utilisation qui est faite des définitions révisées dans les deux Codes, s'il y a lieu.

Les deux Commissions ont décidé s'informer mutuellement de leurs propositions d'amendements respectives avant les réunions de février 2023 et de les présenter dans leur rapport de février 2023.

6.2. Maladies émergentes

6.2.1. Infection par le virus de l'œdème de la carpe (CEV)

Des commentaires ont été formulés par le Japon.

Contexte

Lors de sa réunion de février 2020, la Commission des animaux aquatiques a examiné les informations scientifiques sur l'infection par le virus de l'œdème de la carpe. Elle en a conclu que cette dernière répondait à la définition de « maladie émergente » de l'OIE et qu'à ce titre, les Membres devraient déclarer sa présence conformément aux dispositions de l'article 1.1.4. du chapitre 1.1. « Notification des maladies et communication des informations épidémiologiques » du *Code aquatique*.

Lors de ses réunions de février et septembre 2021, la Commission a examiné les commentaires des Membres et passé en revue les données scientifiques les plus récentes sur l'infection par le virus de l'œdème de la carpe. Elle a noté que la présence de l'infection par le virus de l'œdème de la carpe continuait à être rapportée et qu'elle causait des mortalités chez les populations sauvages et d'élevage. Toutefois, la sévérité de conséquences de la maladie demeurait peu précisément documentée

Lors de sa réunion de février 2022, la Commission a examiné les éléments probants scientifiques les plus récents. Elle a constaté que les mortalités causées par l'infection par le virus de l'œdème de la carpe suscitaient de vives inquiétudes au sein de la communauté scientifique et chez les éleveurs de poissons d'ornement, ce qui s'était d'ailleurs traduit par une augmentation du nombre de rapports et d'articles scientifiques publiés chaque année sur cette maladie. La Commission a également indiqué que le séquençage du génome du virus de l'œdème de la carpe publié en 2021 s'avèrerait utile pour faciliter, dans un futur proche, la conduite d'enquêtes épidémiologiques, la réalisation d'analyses phylogénétiques de ce virus ainsi que le développement de nouveaux tests de diagnostic de la maladie. La Commission a rappelé une nouvelle fois aux Membres que toute détection de ce virus devait être rapportée à l'OMSA au titre de maladie émergente, conformément à l'article 1.1.4. du *Code aquatique*.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapports de février 2020 (point 7.3.3., page 17), septembre 2020 (point 6.3., page 17), février 2021 (partie B : point 2.2., page 11), septembre 2021 (point 5.2.1.1., page 27) et février 2022 (partie B : point 2.2.1.1., page 6).

Réunion de septembre 2022

La Commission a examiné les nouveaux éléments probants scientifiques disponibles sur l'infection par le virus de l'œdème de la carpe. Elle a constaté que, depuis sa réunion de février 2022, l'apparition de plusieurs autres foyers de la maladie avait été rapportée dans la région Asie-Pacifique. La Commission a reconnu que des incertitudes demeuraient quant à l'impact et l'étendue de la propagation de la maladie au niveau mondial, et en particulier en Europe. Pour cette raison, la Commission a souligné qu'il était important que les Membres rapportent les nouvelles détections de l'infection par le virus de l'œdème de la carpe au titre de maladie émergente, afin d'assurer le recueil des informations épidémiologiques et de demeurer vigilant face à la propagation éventuelle de la maladie.

La Commission est convenue que l'infection par le virus de l'œdème de la carpe satisfaisait toujours à la définition de maladie émergente de l'OMSA. Une nouvelle fois, la Commission a demandé aux Membres de bien vouloir lui communiquer tous renseignements pertinents sur l'infection par le virus de l'œdème de la carpe pour lui permettre de vérifier que les critères d'inclusion dans la Liste des maladies (chapitre 1.2.) s'appliquaient ou de conclure que la maladie ne devrait plus être considérée comme une maladie émergente.

La Commission a rappelé aux Membres qu'une fiche technique dédiée à l'infection par le virus de l'œdème de la carpe avait été élaborée et qu'elle était disponible sur le site internet de l'OMSA : https://www.woah.org/fr/ce-que-nous-faisons/sante-et-bien-etre-animale/maladies-animales/.

6.2.2. Infection par le nodavirus CMNV

Contexte

La Commission des animaux aquatiques a prévu, dans son plan de travail, d'examiner les informations scientifiques sur les maladies nouvelles ou émergentes afin de déterminer si elle devait prendre des mesures. Cet examen peut être réalisé à l'initiative de la Commission ou à la demande du Siège de l'OMSA, des Centres

de référence de l'OMSA, d'une représentation régionale de l'OMSA, de groupes *ad hoc* ou de Membres. L'examen des maladies nouvelles ou émergentes est réalisé à chacune des réunions de la Commission.

Réunion de septembre 2022

La Commission a examiné les informations disponibles sur l'infection par le nodavirus CMNV afin de déterminer si elle satisfaisait à la définition de maladie émergente de l'OMSA. Elle a noté que le nombre d'espèces sensibles était extrêmement important et que le virus pouvait infecter et causer la maladie chez les crustacés [par exemple, la crevette pattes blanches (*Penaeus vannamei)* et le bouquet géant (*Macrobrachium rosenbergii*)] et les poissons [par exemple, le tambour à gros yeux *(Larimichthys crocea*), le cardeau hirame (*Paralichthys olivaceus*) et le poisson zèbre (*Danio rerio*)].

La Commission a noté que les rapports sur l'apparition du nodavirus CMNV précisaient qu'il avait été détecté en Asie. La Commission a constaté que l'infection par le nodavirus CMNV (chez les crustacés) était listée sous la dénomination « Viral covert mortality disease » comme maladie des crustacés à déclaration obligatoire par le Réseau des centres d'aquaculture dans la région Asie-Pacifique (NACA) dans son rapport trimestriel sur les maladies des animaux aquatiques (région Asie-Pacifique), depuis 2017.

Des épisodes de mortalités et leurs impacts sur la production en lien avec l'infection par le nodavirus CNMV ont été rapportés. La présence de particules virales dans les gonades indiquerait une possible transmission verticale du virus.

La Commission a conclu que l'infection par le nodavirus CMNV satisfaisait à la définition de maladie émergente et, qu'à ce titre, elle devait être rapportée à l'OMSA conformément à l'article 1.1.4. du *Code aquatique*. La Commission est convenue d'élaborer une fiche technique sur l'infection par le nodavirus CMNV, qui sera publiée sur le site internet de l'OMSA dans les prochains mois.

6.3. E-Certification

Le Secrétariat a informé la Commission des animaux aquatiques que bien que l'adoption par les Membres de la certification électronique soit encore limitée, l'usage de systèmes électroniques allait croissant. Le Secrétariat a également fait un point à la Commission sur les actions engagées par l'OMSA, notamment un projet du Fonds pour l'application des normes et le développement du commerce (STDF) sur la certification vétérinaire électronique. Le projet visait à disposer d'une meilleure compréhension de la façon dont certains Membres, tant développés qu'en voie de développement, mettaient en œuvre la certification vétérinaire électronique et des travaux pertinents conduits par d'autres organisations internationales sur la certification électronique et le guichet unique.

L'OMSA a considéré qu'elle devait envisager des orientations similaires à celles élaborées par le Codex Alimentarius pour les raisons suivantes : l'alignement des travaux de l'OMSA et du Codex Alimentarius (en matière de denrées d'origine animale) ; le fait qu'en pratique, un seul certificat d'exportation peut contenir les informations relatives à la sécurité sanitaire et à la santé des animaux aquatiques ; l'adoption, en 2021, par le Codex Alimentarius de la version révisée des Directives pour la conception, l'établissement, la délivrance et l'utilisation des certificats officiels génériques (CXG 38-2001), qui traitent spécifiquement de la transition vers l'échange dématérialisé de certificats officiels.

La Commission a été informée que l'OMSA développerait des modèles de référence des données (versions électroniques) pour les modèles de certificats sanitaires relatifs au commerce international figurant au chapitre 5.11. du *Code aquatique*. Ces modèles de référence des données seront similaires à celui utilisé pour le modèle de référence générique de certificat officiel qui figure dans les Directives susmentionnées du *Codex Alimentarius*.

Pour l'ensemble des raisons invoquées ci-après, la Commission a décidé d'inclure la révision du chapitre 5.2. dans son plan de travail : l'introduction de la certification électronique peut contribuer à faciliter les échanges commerciaux internationaux et limiter le risque de fraudes commerciales ; en outre les perturbations des échanges causées par la pandémie de COVID-19 ont souligné l'intérêt que représente cette approche. La Commission a insisté sur l'importance de travailler en étroite collaboration avec la Commission du Code puisque le *Code terrestre* comporte un chapitre similaire.

Manuel des tests de diagnostic pour les animaux aquatiques de l'OMSA

7. Textes soumis aux Membres pour avis

La Commission des animaux aquatiques s'est engagée dans un processus de reformatage progressif des chapitres spécifiques aux maladies du *Manuel aquatique* selon le nouveau modèle de chapitre. Étant donné que les chapitres reformatés et les chapitres mis à jour ont subi des modifications substantielles, la Commission a décidé, lors de sa réunion de septembre 2019, que seules les versions exemptes des marques de révision seraient présentées dans le présent rapport. Les modifications apportées ultérieurement au projet de révision initial pour prendre en compte les commentaires des Membres seraient indiquées de la façon usuelle (c'est-à-dire par l'utilisation des fonctions « double souligné » et « ~~barré~~ » pour signaler respectivement les ajouts et les suppressions).

Un document comparant le texte de la version adoptée d'un chapitre au texte de la proposition de nouveau modèle de ce chapitre peut être généré par un logiciel informatique. Ce document n'est pas inclus dans le rapport de la Commission mais sera disponible sur demande auprès du Service des normes de l'OMSA (AAC.secretariat@woah.org).

Lors de l'examen des commentaires des Membres et des chapitres nouvellement mis à jour, la Commission a noté que certains amendements étaient applicables à l'ensemble des chapitres. Elle a donc décidé d'amender le modèle et d'appliquer les modifications suivantes à l'ensemble des chapitres concernés ; elle a :

1. ajouté, dans la cellule concernée de la colonne répertoriant les méthodes du Tableau 4.1. « OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals », le terme « Conventional PCR followed by » avant le terme « amplicon sequencing », afin de rappeler qu'aux fins du diagnostic de confirmation, l'étape de séquençage de l'amplicon impliquait nécessairement une étape préalable de PCR (polymerase chain reaction). De même, pour rappeler qu'aux fins d'une confirmation de cas, la technique de PCR conventionnelle ne devait jamais être utilisée sans qu'un séquençage soit également réalisé, les cellules de la ligne « Conventional PCR » ont été colorées en gris au niveau des colonnes de la Section C « Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis ».

2. ajouté, dans la section 6. « Corroborative diagnostic criteria », le modèle de texte suivant à la fin du second paragraphe :

   « . . . It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the WOAH Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for testing. »

   Comme conséquence de cet ajout, le paragraphe suivant a été supprimé de la section 6.1.2. « Definition of confirmed case in apparently healthy animals » et 6.2.2. « Definition of confirmed case in clinically affected animals » :

   « ~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

3. remplacé, dans la deuxième phrase du premier paragraphe de la section 6.1. « Apparently healthy animals or animals of unknown health status », le terme « Geographical » par le terme « Hydrographical »:

   « Hydrographical ~~Geographical~~ proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. »

La Commission a examiné le texte de l'Introduction générale de la partie 2. « Recommandations applicables à des maladies spécifiques » du *Manuel aquatique*, qui a été adoptée en 2012 et présente une approche globale en matière de gestion, de surveillance, d'échantillonnage en santé des animaux aquatiques. La Commission a relevé qu'une partie du texte était devenue obsolète ou contrevenait au contenu de chapitres adoptés plus récemment, comme par exemple le chapitre 1.1.1. « Gestion de la qualité dans les laboratoires de diagnostic vétérinaire ». La Commission a décidé de recommander le retrait de ce chapitre du *Manuel aquatique* car il n'est désormais plus pertinent ou adapté au regard de l'objectif poursuivi.

La Commission a invité les Membres à formuler des commentaires sur le retrait de l'Introduction générale de la partie 2. « Recommandations applicables à des maladies spécifiques » du *Manuel aquatique*. Aucune annexe n'est fournie sur ce point puisque c'est le retrait de l'intégralité du chapitre qui est proposé.

7.1. Section 2.2. « Maladies des crustacés »

7.1.1. Chapitre 2.2.0. « Informations générales (Maladies des crustacés) »

Réunion de septembre 2022

Le chapitre 2.2.0. « Informations générales (Maladies des crustacés) » a été mis à jour avec le concours des experts des Laboratoires de référence sur les crustacés.

Parmi les principaux amendements figurent :

- la suppression des deux paragraphes de la section A.1. « Assessing the health status of the epidemiological unit » parce que les informations présentées n'ont pas été jugées utiles et qu'aucun texte similaire ne figurait dans le chapitre 2.3.0. « Information générale (Maladies des poissons) » ;
- le réordonnancement des sections figurant la section A. « Sampling » aux fins de l'alignement de la structure du chapitre sur celle du chapitre 2.3.0. ;
- dans un souci de clarté, la mise à jour du texte sur la sélection des échantillons d'animaux figurant dans la section A.1.2. « Specifications according to crustacean populations » ;
- la mise à jour complète du texte figurant dans les sections B.5.5 « Use of molecular and antibody-based techniques for confirmatory testing and diagnosis », B.5.5.3. « Nucleic acid extraction » et B.5.5.4. « Preparation of slides for *in-situ* hybridisation » ;
- le développement de la section B.6. « Additional information to be collected », afin d'y insérer le texte sur les antécédents des spécimens ;
- la mise à jour des références.

La version révisée du chapitre 2.2.0. « Informations générales (Maladies des crustacés) » est présentée aux Membres en Annexe 17 afin qu'ils formulent leurs commentaires.

7.1.2. Chapitre 2.2.1. « Maladie de nécrose hépatopancréatique aiguë »

Des commentaires ont été formulés par l'Australie, le Canada, la Chine (Rép. populaire de), les États-Unis d'Amérique, le Taipei chinois, la Norvège, la Suisse et l'UE.

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a examiné le chapitre 2.2.1. « Maladie de nécrose hépatopancréatique aiguë », qui a été mis à jour par les experts du Laboratoire de référence et reformaté selon le nouveau modèle de chapitre. La version révisée a été présentée aux Membres pour avis dans la partie B du rapport de février 2022 de la Commission.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partie B : point 3.1.1.1., page 9).

Réunion de septembre 2022

Selon le champ d'application du chapitre, l'expression « maladie de nécrose hépatopancréatique aiguë » désigne une infection causée par des souches de *Vibrio parahaemolyticus* (V_{pAHPND}), porteuses d'un plasmide de ~70-kbp, dont les gènes codent pour des toxines homologues aux toxines entomopathogènes de *Photorhabdus* (Pir), PirA et PirB. Un Membre a demandé que le champ d'application du chapitre soit étendu afin de prendre en compte les cas rapportés de la maladie lorsque celle-ci est causée par d'autres espèces de *Vibrio*. La Commission, avec le concours des Laboratoires de référence de l'OMSA, examinera les informations publiées sur les espèces autres que *Vibrio parahaemolyticus* et qui ont été associées avec des cas de maladie de nécrose hépatopancréatique aiguë. Elle présentera ses conclusions lors de sa réunion de février 2023.

La Commission est convenue de supprimer le texte figurant dans la section 2.2.5. « Aquatic animal reservoirs of infection » et de le remplacer par la mention « aucun connu ». En effet, le texte fait référence à des études expérimentales mais ne confirme pas l'existence de réservoirs de l'infection (animaux infectés mais non symptomatiques qui peuvent transmettre la maladie).

En réponse à une demande de clarification sur la phrase suivante de la section 2.3.1. « Mortality, morbidity and prevalence » : « "In regions where AHPND is enzootic in farmed shrimp, evidence indicates a near 100% prevalence (Tran *et al.*, 2014) », la Commission a confirmé que la prévalence concernait les espèces de *Vibrio parahaemolyticus* responsables de la maladie et porteurs des gènes codant pour les toxines PirA et PirB.

La Commission a accepté la proposition de transférer le texte sur les lésions macroscopiques de la section 2.3.2. « Clinical signs, including behavioural changes » vers la section 2.3.3. « Gross pathology » et de

supprimer le texte sur les lésions microscopiques tissulaires et cellulaires de la section 2.3.3., car il figure déjà dans la section 4.2. « Histopathology and cytopathology ».

Dans la section 3.2. « Selection of organs or tissues », la Commission a accepté de supprimer le texte sur le prélèvement des échantillons de fèces car il figure déjà dans la section 3.4. « Non-lethal sampling ». La Commission a également accepté de supprimer le texte figurant dans la section 3.5. « Preservation of samples for submission », car il figure déjà dans les sections 3.5.1. « Samples for pathogen isolation », 3.5.2. « Preservation of samples for molecular detection » et 3.5.3. « Samples for histopathology, immunohistochemistry or *in-situ* hybridisation ».

Dans la section 3.5.2. « Preservation of samples for molecular detection », pour plus de clarté, la Commission a complété la phrase « If material cannot be fixed it may be frozen », en y ajoutant « but repeated freezing and thawing of samples should be avoided ».

Dans le tableau 4.1. « OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals », la Commission a décidé, aux fins de l'objectif C. « Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis' », d'attribuer la note « ++ » à la PCR en temps réel pour l'ensemble des stades de développements des espèces hôtes et lui a attribué un niveau de validation égal à « 1 ». Cette modification est en adéquation avec la définition de cas figurant dans la section 6. « Corroborative diagnostic criteria » et permet d'augmenter à « 2 » le niveau de validation attribué à la PCR conventionnelle suivi d'un séquençage de l'amplicon, qui est incluse dans le tableau de la section 6.3.1. « For presumptive diagnosis of clinically affected ». Après avoir consulté les experts du Laboratoire de référence, la Commission a décidé d'ajouter la méthode LAMP (loop-mediated isothermal amplification) dans le tableau aux fins de l'objectif A. « Surveillance of apparently healthy animals » ainsi que la méthode ELISA aux fins des trois objectifs. Les modifications du Tableau 4.1. décrites au point 7 de l'ordre du jour ci-dessus ont également été apportées.

La Commission n'a pas accepté la demande de suppression de la section 4.2. « Histopathology and cytopathology », car l'information y figurant est essentielle et non présentée dans d'autres sections du chapitre.

En réponse à une demande de clarification d'une phrase de la section 4.4. « Nucleic acid amplification » sur le nombre d'isolats utilisés dans l'étude de validation de la méthode AP3 PCR, la Commission a décidé de supprimer cette partie de la phrase prêtant à confusion ; il est possible de disposer des détails de l'étude dans la publication à laquelle il est fait référence. De même, dans la section 4.4., un Membre a indiqué qu'il existait des méthodes sensibles reposant sur des techniques LAMP et de PCR nichée. Il a demandé qu'elles soient incluses dans le tableau 4.1. ou que les raisons pour lesquelles elles n'étaient pas recommandées soient fournies. Le Membre a également indiqué que la section 4.9. « Antibody- or antigen-based detection methods » faisait référence à une méthode sensible et spécifique reposant sur une technique Ag-ELISA (antigen enzyme-linked immunosorbent assay) mais que cette dernière ne figurait pas dans le tableau 4.1. Après consultation des experts des Laboratoires de référence, la Commission a jouté les méthodes LAMP et Ag-ELISA dans le tableau 4.1.

Dans la section 4.4. « Nucleic acid amplification », la Commission a ajouté le texte standard sur les témoins et l'extraction des acides nucléiques, ainsi que les tableaux répertoriant les amorces, les sondes ainsi que les paramètres des cycles pour la PCR en temps réel et pour la PCR conventionnelle, aux fins de la détection des gènes de Vp_{AHPND} codant pour les toxines, tel que détaillé au point 7.4. ci-après de l'ordre du jour. L'inclusion des tableaux types a permis de supprimer certains détails des protocoles de PCR.

Dans la section 4.4.2. « Conventional PCR, Protocol for the AP1 and AP2 PCR methods », la Commission a ajouté à la liste de références le lien hypertexte valide permettant d'accéder au protocole.

S'agissant de la section 5. « Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations », un Membre a constaté que la PCR en temps réel et la PCR conventionnelle avaient des notes d'utilisation et des niveaux de validation identiques. Il a donc proposé que la PCR conventionnelle soit également mentionnée dans la section 5. Après consultation des Laboratoires de référence, la PCR conventionnelle a été introduite dans la section 5.

La Commission a amendé les définitions de cas figurant dans la section 6. « Corroborative diagnostic criteria » afin qu'elles soient en ligne avec les modifications apportées au tableau 4.1. La Commission a également procédé aux modifications génériques de la section 6 décrites au point 7 de l'ordre du jour ci-dessus.

La version révisée du chapitre 2.2.1. « Maladie de nécrose hépatopancréatique aiguë » est présentée en Annexe 18 afin que les Membres formulent leurs commentaires.

7.1.3. Chapitre 2.2.2. « Infection à *Aphanomyces astaci* (peste de l'écrevisse) »

Réunion de septembre 2022

La Commission des animaux aquatiques a examiné le chapitre 2.2.2. « Infection à *Aphanomyces astaci* (peste de l'écrevisse) », qui a été mis à jour par les experts du Laboratoire de référence et reformaté selon le nouveau modèle de chapitre.

Parmi les principaux amendements figurent :

- la mise à jour de l'information sur l'agent étiologique ; étant donné que le groupe *ad hoc* sur la sensibilité des espèces de crustacés à une infection par une maladie listée par l'OIE n'a pas réalisé les évaluations, la version actuelle du texte figurant dans les sections 2.2.1. « Susceptible host species » et 2.2.2. « Species with incomplete evidence for susceptibility » demeure inchangée ;
- la mise à jour de l'information dans les sections sur les caractéristiques de la maladie, sur les stratégies en matière de sécurité biologique et de contrôle des maladies, sur la sélection des spécimens ainsi que sur le prélèvement, le transport et la manipulation des échantillons ;
- la mise à jour de l'information dans la section 4. « Diagnostic methods », notamment l'insertion des modifications décrites au point 7 de l'ordre du jour et la complétude du Tableau 4.1. « OIE Recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals » ;
- la suppression des descriptions détaillées figurant dans la section 4.3. « Culture for isolation », car il ne s'agit pas d'une méthode de diagnostic utilisée en routine mais plutôt d'une méthode utilisée en recherche ou pour le maintien des cultures ;
- la mise à jour de la section 4.4. « Nucleic acid amplification », notamment par l'ajout du texte standard tel que décrit au point 7 de l'ordre du jour ci-dessus et relatif aux témoins et à l'extraction des acides nucléiques pour les PCR, ainsi qu'aux tableaux types répertoriant les amorces, sondes et paramètres des cycles. L'inclusion des tableaux types a permis de supprimer certains détails des protocoles de PCR ;
- la mise à jour de la section 4.5. « Amplicon sequencing », notamment par l'ajout du texte standard (voir le point 7 de l'ordre du jour), et
- la révision des définitions de cas suspect et de cas confirmé chez les animaux apparemment sains et présentant des signes cliniques.

La version révisée du chapitre 2.2.2. « Infection à *Aphanomyces astaci* (peste de l'écrevisse) » est présentée en Annexe 19 afin que les Membres formulent leurs commentaires.

7.1.4. Chapitre 2.2.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) »

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a examiné le chapitre 2.2.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) », qui avait été mis à jour par les experts du Laboratoire de référence et reformaté selon le nouveau modèle de chapitre. La version révisée avait été présentée aux Membres pour avis dans la partie B du rapport de février 2022 de la Commission.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partie B : point 3.1.1.2., page 10).

Réunion de septembre 2022

La Commission a mis à jour la description taxonomique figurant dans la section 1. « Scope » afin qu'elle soit en ligne avec le chapitre 9.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) » du *Code aquatique*. Elle a inclu une référence à la famille des Holosporaceae et à l'ordre des Rickettsiales afin de prendre en compte la classification taxonomique reconnue.

La Commission a accepté de transférer le texte sur les mortalités de la section 2.3.3. « Gross pathology » vers la section 2.3.1. « Mortality, morbidity and prevalence ». En réponse à une demande de détails sur les reproducteurs affectés par l'hépatopancréatite nécrosante et touchés par des mortalités, la Commission a indiqué sue l'information figurait dans la référence fournie et a choisi de ne pas la faire figurer dans le chapitre.

La Commission est convenue de supprimer le texte figurant dans les sections 2.3.2. « Clinical signs, including behavioural changes » et 2.3.3. « Gross pathology » qui n'était pas pertinent au regard des sujets traités respectivement par ces deux sections.

Dans la section 2.4. « Biosecurity and disease control strategies », la Commission a accepté de supprimer la dernière phrase sur la sensibilité de *H. penaei*, et qui reposait sur une référence publiée en 1994.

Dans la section 3.2. « Selection of organs or tissues », la Commission a précisé que l'hépatopancréas, principal organe ciblé par l'agent pathogène, devait être sélectionné de façon préférentielle.

La Commission a révisé la section 3.4. « Non-lethal sampling » afin d'actualiser le contenu et d'améliorer le texte.

Dans la section 3.5.2. « Preservation of samples for molecular detection », pour plus de clarté, la Commission a complété la phrase « If material cannot be fixed it may be frozen » en y ajoutant à la fin « but repeated freezing and thawing of samples should be avoided ».

Dans le tableau 4.1. « OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals », la Commission a modifié, aux fins de l'objectif A. « Surveillance of apparently healthy animals' », la note de la PCR conventionnelle de « +++ » en « ++ » car ce test n'est pas adapté pour la surveillance. Les modifications du tableau 4.1. décrites au point 7 de l'ordre du jour ci-dessus ont également été apportées

Dans la section 4.4. « Nucleic acid amplification », la Commission a ajouté le texte standard sur les témoins pour la PCR et l'extraction des acides nucléiques, ainsi que les tableaux répertoriant les amorces, sondes et paramètres des cycles pour la PCR en temps réelle et pour la PCR conventionnelle aux fins de la détection de *H. penaei,* tel que détaillé au point 7.4. de l'ordre du jour ci-après. L'inclusion des tableaux types a permis de supprimer certains détails des protocoles de PCR. Les modifications du tableau 4.1. décrites au point 7 de l'ordre du jour ci-dessus ont également été apportées

Dans la section 4.5. « Amplicon sequencing », le texte standard sur le séquençage de l'amplicon a remplacé le texte existant.

La Commission n'a pas accepté d'inclure la PCR conventionnelle en plus de la PCR en temps réel dans la section 5. « Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations », car la PCR conventionnelle n'est pas idéale pour la surveillance. La Commission a corrigé la note attribuée à la PCR conventionnelle dans le 4.1. comme mentionné ci-dessus.

Dans la section 6. « Corroborative diagnostic criteria », la Commission a procédé aux modifications génériques décrites au point 7 de l'ordre du jour ci-dessus.

La version révisée du chapitre 2.2.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) » est présentée en Annexe 20 afin que les Membres formulent leurs commentaires.

7.1.5. Chapitre 2.2.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuses »

Des commentaires ont été formulés par l'Australie, la Chine (Rép. populaire de), les États-Unis d'Amérique, la Norvège, la Suisse et l'UE.

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a examiné le chapitre 2.2.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuses », qui avait été mis à jour par les experts du Laboratoire de référence et reformaté selon le nouveau modèle de chapitre. La version révisée a été présentée aux Membres pour avis dans la partie B du rapport de février 2022 de la Commission.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partie B : point 3.1.1.3., page 10).

Réunion de septembre 2022

Dans la section 1. « Scope », la Commission a mis à jour la classification taxonomique du virus afin qu'il soit en ligne avec celle figurant dans le chapitre 9.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse ».

Dans la section 1. « Aetiological agent », la Commission a ajouté une référence démontrant que deux génotypes distincts du virus de la nécrose hypodermique et hématopoïétique infectieuse (type 1 et type 2) pouvaient infecter *Penaeus vannamei* et *P. monodon*. La Commission a également corrigé la phrase précisant la région dans laquelle le type 1 a été identifié : il ne s'agit pas de l'Asie du Sud mais de l'Asie du Sud-Est.

Dans la section 2.2.2. « Species with incomplete evidence for susceptibility », la Commission est convenue de supprimer la phrase suivante: « ~~Evidence is lacking for this species to either confirm that the identity of the pathogenic agent is IHHNV, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection~~». En effet, sa présence est la conséquence d'un oubli de suppression dans une précédente version du chapitre et n'est désormais plus en adéquation avec le modèle. Par ailleurs, toujours dans la section 2.2.2., la Commission n'a pas accepté de supprimer le texte introductif figurant dans le second paragraphe, « pathogen-specific positive PCR results had been reported in the following organisms, but an active infection has not been demonstrated : ». La Commission a en effet estimé que ce texte explicatif, qui figure dans le modèle, était important, car il reprenait les résultats des travaux du groupe *ad hoc* sur la sensibilité des espèces de crustacés à une infection par une maladie listée par l'OIE.

La Commission a accepté la proposition de transférer une partie du texte figurant dans la section 2.3.3. « Gross pathology » dans la section 2.3.1. « Mortality, morbidity and prevalence » ainsi que de déplacer le troisième paragraphe de la section 2.3.1. dans la section 2.3.2. « Clinical signs, including behavioural changes » car les textes concernés y seront plus en adéquation. Dans la section 2.3.1., la Commission a ajouté un nouveau texte ainsi qu'une référence sur l'absence de signes cliniques et les mortalités chez les animaux infectés de façon expérimentale par des génotypes du virus de la nécrose hypodermique et hématopoïétique infectieuse appartenant à une lignée distincte des génotypes de type II circulant en Équateur et au Pérou. Finalement, la Commission a ajouté un nouveau texte dans la section 2.3.2. afin de préciser certains des signes cliniques et modifications comportementales causés par l'infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse.

Dans le tableau 4.1. « OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals », la Commission a supprimé, aux fins de l'objectif C. « Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis' », la note attribuée à l'histopathologie, car cette méthode n'est pas suffisamment spécifique pour la confirmation d'un cas. Les modifications du tableau 4.1. décrites au point 7 de l'ordre du jour ci-dessus ont également été apportées

Dans la section 4.4. « Nucleic acid amplification », la Commission a ajouté le texte standard sur les témoins pour la PCR et l'extraction des acides nucléiques, ainsi que les tableaux répertoriant les amorces, sondes et paramètres des cycles pour la PCR en temps réel et pour la PCR conventionnelle aux fins de la détection de l'infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse, tel que détaillé au point 7.4. de l'ordre du jour ci-après. L'inclusion des tableaux types a permis de supprimer certains détails des protocoles de PCR.

Dans la section 4.5. « Amplicon sequencing », le texte standard sur le séquençage de l'amplicon a remplacé le texte existant (voir le point 7.4. à l'ordre du jour ci-après).

Dans la section 6. « Corroborative diagnostic criteria », la Commission a procédé aux modifications génériques décrites au point 7 de l'ordre du jour ci-dessus.

La Commission a modifié le texte figurant dans les sections 6.1.2. « Definition of confirmed case in apparently healthy animals » et 6.2.2. « Definition of confirmed case in clinically affected animals » afin qu'il soit en ligne avec celui des autres chapitres. Elle a en outre supprimé l'histopathologie car cette méthode n'est pas suffisamment spécifique pour la confirmation d'un cas.

La version révisée du chapitre 2.2.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse » est présentée en Annexe 21 afin que les Membres formulent leurs commentaires.

7.1.6. Chapitre 2.2.5. « Infection par le virus de la myonécrose infectieuse »

Réunion de septembre 2022

La Commission des animaux aquatiques a examiné le chapitre 2.2.5. « Infection par le virus de la myonécrose infectieuse ». En raison de l'absence de Laboratoire de référence pour cette maladie, le chapitre a été reformaté selon le nouveau modèle de chapitre et passé en revue par la Commission.

Parmi les principaux amendements figurent :

- la mise à jour de l'information sur la taxonomie figurant dans le champ d'application du chapitre et sur l'agent étiologique ;
- la mise à jour des sections sur les vecteurs, la mortalité, la morbidité et la prévalence, la distribution géographique et la chimiothérapie, notamment les agents inhibiteurs ;
- la mise à jour de la section relative aux méthodes de diagnostic, par la complétude du tableau 4.1. et par la révision des sections sur la culture cellulaire pour l'isolement du virus, l'amplification de l'acide nucléique et les tests moléculaires, notamment en ajoutant le texte standard sur les témoins pour la PCR et l'extraction des acides nucléiques, le séquençage de l'amplicon et le remplacement des protocoles de tests par de nouveaux tableaux répertoriant les amorces, sondes et paramètres des cycles de PCR ;
- la révision des définitions de cas suspect et de cas confirmé chez les animaux apparemment sains et présentant des signes cliniques, et
- la complétude du tableau figurant dans la section sur la spécificité et la sensibilité des tests de diagnostic destinés au diagnostic présumé des animaux présentant des signes cliniques.

La version révisée du chapitre 2.2.5. « Infection par le virus de la myonécrose infectieuse » est présentée en Annexe 22 afin que les Membres formulent leurs commentaires.

7.1.7. Chapitre 2.2.7. « Infection par le virus du syndrome de Taura »

Réunion de septembre 2022

La Commission des animaux aquatiques a examiné le chapitre 2.2.7. « Infection par le virus du syndrome de Taura », qui a été mis à jour par les experts du Laboratoire de référence et reformaté selon le nouveau modèle de chapitre.

Parmi les principaux amendements figurent :

- la mise à jour des informations sur la distribution de l'agent pathogène dans l'hôte, la distribution géographique, la production de souches résistantes et l'échantillonnage non létal ;
- la mise à jour de l'information relative aux méthodes de diagnostic, par la complétude du tableau 4.1. et par la révision des sections sur le dosage biologique, l'amplification de l'acide nucléique et les tests moléculaires, notamment en ajoutant le texte standard sur les témoins pour la PCR, l'extraction des acides nucléiques et le séquençage de l'amplicon, et, enfin le remplacement des protocoles de tests par de nouveaux tableaux répertoriant les amorces, sondes et paramètres des cycles de PCR ;
- la révision des définitions de cas suspect et de cas confirmé chez les animaux apparemment sains et présentant des signes cliniques, et
- la mise à jour des références.

La version révisée du chapitre 2.2.7. « Infection par le virus du syndrome de Taura » est présentée en Annexe 23 afin que les Membres formulent leurs commentaires.

7.1.8. Chapitre 2.2.8. « Infection par le virus du syndrome des points blancs »

Réunion de septembre 2022

La Commission des animaux aquatiques a examiné le chapitre 2.2.8. « Infection with white spot syndrome virus », qui a été mis à jour par les experts du Laboratoire de référence et reformaté selon le nouveau modèle de chapitre.

Parmi les principaux amendements figurent :

- la mise à jour de l'information sur l'agent étiologique ;
- la mise à jour de l'information dans les sections sur la survie et la stabilité dans les échantillons traités ou entreposés, les réservoirs de l'infection parmi les animaux aquatiques, les vecteurs, la distribution géographique, les méthodes d'inactivation ;

- la mise à jour de l'information figurant dans la section relative aux méthodes de diagnostic, par la complétude du tableau 4.1. et par la révision de la section sur l'amplification de l'acide nucléique et les tests moléculaires, notamment en ajoutant le texte standard sur les témoins pour la PCR, l'extraction des acides nucléiques et le séquençage de l'amplicon, et, enfin en remplaçant les informations sur les protocoles de tests par de nouveaux tableaux répertoriant les amorces, sondes et paramètres des cycles de PCR ;
- la révision des définitions de cas suspect et de cas confirmé chez les animaux apparemment sains et présentant des signes cliniques ;
- la complétude du tableau figurant dans la section sur la spécificité et la sensibilité des tests de diagnostic destinés, et
- la mise à jour des références.

La Commission a noté que les résultats des évaluations réalisées par le groupe *ad hoc* sur la sensibilité des espèces de crustacés à une infection par une maladie listée par l'OIE, en juin 2016, pour l'infection par le virus du syndrome des points blancs, n'ont pas été utilisés par la Commission car l'article 1.5.9. du chapitre 1.5. « Critères d'inclusion dans la liste des espèces sensibles à une infection par un agent pathogène spécifique » n'avait pas encore été adopté. Ces évaluations seront examinées plus en détail par la Commission aux fins de l'application de l'article 1.5.9. La Commission a indiqué que le texte actuellement en vigueur demeurerait inchangé dans la section 2.2.1. tant que ses travaux sur ce point ne seraient pas achevés.

Pour plus de détails sur les évaluations conduites par le groupe *ad hoc*, la Commission a invité les membres à consulter le rapport de juin 2016, disponible sur le site internet de l'OMSA.

La version révisée du chapitre 2.2.8. « Infection par le virus du syndrome des points blancs » est présentée en Annexe 24 afin que les Membres formulent leurs commentaires.

7.2. Section 2.3. « Maladies des poissons »

7.2.1. Chapitre 2.3.1. « Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique) »

Des commentaires ont été formulés par la Chine (Rép. populaire de), les États-Unis d'Amérique, la Thaïlande et l'UE.

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a examiné le chapitre 2.3.1. « Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique) », qui a été mis à jour par les experts du Laboratoire de référence de l'OMSA et reformaté selon le nouveau modèle de chapitre. La version révisée du chapitre a été présentée aux Membres dans la partie B du rapport de février 2022 de la Commission afin qu'ils formulent leurs commentaires.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partie B : point 3.1.2.1., page 10).

Réunion de septembre 2022

La Commission a supprimé le terme « fungus » après le terme « oomycete » dans la section 1. « Scope » car les oomycètes ont été transférés du règne des champignons vers le règne des protozoaires.

La Commission a ajouté un commentaire sur les noms vernaculaires listés dans les tableaux de la section 2.2.1. « Susceptible host species » car cette dernière fait actuellement l'objet d'une révision par le groupe *ad hoc* sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE. Une fois que la liste des espèces aura été finalisée par le groupe *ad hoc*, une vérification de leurs noms vernaculaires au regard de la base de données FAO sera réalisée.

Dans la section 3.6. « Pooling of samples », la Commission est convenue d'amender le texte en y insérant la phrase de recommandation suivante : « The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger specimens should be processed and tested individually. ». Le texte est désormais en ligne avec celui du modèle et, par conséquent, avec les autres chapitres.

Dans le tableau 4.1. « OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals », la Commission a ajouté une

nouvelle ligne relative aux signes cliniques et lui a attribué des notes pour les trois objectifs. La Commission a également ajouté des notes aux « Squash mounts » aux fins de l'objectif C. « Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis' ». Les modifications du tableau 4.1. décrites au point 7 de l'ordre du jour ci-dessus ont également été apportées.

Dans la section 4.4. « Nucleic acid amplification », la Commission a ajouté le texte standard sur les témoins et l'extraction des acides nucléiques, ainsi que les tableaux répertoriant les amorces, sondes et paramètres des cycles pour la PCR en temps réelle et pour la PCR conventionnelle, aux fins de la détection de *A. invadans* dans les tissus des poissons, tel que détaillé au point 7.4. ci-après de l'ordre du jour. L'inclusion des tableaux types a permis de supprimer certains détails des protocoles de PCR.

Dans la section 6. « Corroborative diagnostic criteria », la Commission a décidé de ne pas inclure le texte standard relatif à l'envoi des échantillons prélevés sur des animaux suspects au Laboratoire de référence de l'OMSA car, actuellement, il n'y en a aucun. Dans le cas où un Laboratoire de référence de l'OMSA devait être désigné pour le syndrome ulcératif épizootique, le texte serait alors ajouté dans la section.

Dans la section 6.1.1. « Definition of suspect case in apparently healthy populations », la Commission n'a pas accepté de supprimer le texte relatif à la présence de signes cliniques évocateurs de l'infection à *A. invadans* car la recherche de ces signes cliniques est recommandée, dans la section 5. « Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations », comme méthode de surveillance des populations apparemment en bonne santé. De plus, les signes cliniques peuvent être non pathognomoniques et satisfaire par conséquent aux critères de la définition de cas suspect. La Commission a ajouté une note de bas de page pour ce critère expliquant que la surveillance de populations apparemment en bonne santé repose, pour le syndrome ulcératif épizootique, sur la recherche de signes cliniques évocateurs de l'infection à *A. invadans* dans la population cible.

Dans la section 6.2.1. « Definition of suspect case in clinically affected animals », la Commission a décidé de préciser les critères iv) et v) de la façon suivante : « Visual observation […] of hyphae characteristic of *A. invadans* » et « Culture and isolation of *A. invadans*-type colonies ».

La version révisée du chapitre 2.3.1. « Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique) » est présentée en annexe 25 afin que les Membres formulent leurs commentaires.

7.2.2. Chapitre 2.3.2. « Infection par le virus de la nécrose hématopoïétique épizootique »

Des commentaires ont été formulés par les États-Unis d'Amérique, la Norvège, la Suisse et l'UE.

Contexte

Lors de sa réunion de septembre 2021, la Commission des animaux aquatiques a examiné le chapitre 2.3.2. « Infection par le virus de la nécrose hématopoïétique épizootique », qui a été mis à jour par les experts du Laboratoire de référence de l'OMSA et reformaté selon le nouveau modèle de chapitre. La version révisée du chapitre a également été présentée aux Membres dans la partie B du rapport de février 2022 de la Commission afin qu'ils formulent leurs commentaires.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapports de septembre 2021 (point 6.1.3., page 31) et février 2022 (partie B : point 3.1.2.2., page 11).

Réunion de septembre 2022

Dans la section 3.6. « Pooling of samples », la Commission n'a pas accepté de supprimer deux phrases sur les procédures de mélange d'échantillons à suivre dans les cas où l'effet du mélange sur la sensibilité du test de diagnostic n'aurait pas été complètement évalué. La Commission a indiqué que ces deux phrases faisaient partie du texte standard destiné à cette section tel que prévu dans le nouveau modèle de chapitre et qu'elles s'inscrivaient dans l'approche visant à établir des recommandations fondées sur des données factuelles. La Commission a toutefois accepté de supprimer la dernière phrase, c'est-à-dire « If pooling is used, it is recommended to pool organ pieces from a maximum of five fish », car sa présence est la conséquence d'un oubli de suppression dans une version plus ancienne du chapitre et n'est désormais plus souhaitée.

Dans le tableau 4.1. « OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals », la Commission a précisé qu'il était essentiel d'indiquer les notes attribuées à la culture cellulaire, la PCR en temps réel et la PCR conventionnelle, même si le séquençage de l'amplicon est requis pour le diagnostic de confirmation. En outre, la Commission a

réduit de « 2 » à « 1 » le niveau de validation attribué à la culture cellulaire aux fins des trois objectifs et celui attribué à la PCR en temps réel aux fins des objectifs A « Surveillance of apparently healthy animals » et C. « Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis' », car aucune donnée n'a été publiée sur la sensibilité et la spécificité de cette méthode de diagnostic, que ce soit pour les animaux apparemment en bonne santé ou ceux présentant une forme clinique de la maladie. Les modifications du tableau 4.1. décrites au point 7 de l'ordre du jour ci-dessus ont également été apportées.

Dans la section 4.3.2. « Cell culture », la Commission, avec le concours de l'expert du Laboratoire de référence, a décidé de supprimer, dans la dernière phrase, la partie suivante : « immunostaining, ELISA, and immuno-electron microscopy » ; elle a révisé le texte afin de préciser que l'identité des virus maintenus sur culture cellulaire était déterminée par « PCR and amplicon sequencing ».

Dans la section 4.4. « Nucleic acid amplification », la Commission, avec le concours de l'expert du Laboratoire de référence, a amendé la première phrase afin de rappeler que, malgré la description de plusieurs méthodes de PCR conventionnelle ou PCR quantitative en temps réel utilisées pour la détection de ranavirus, le virus de la nécrose hématopoïétique épizootique pouvait uniquement être détecté en combinant ces méthodes avec celles permettant de le détecter de façon spécifique. La Commission a également accepté de supprimer une phrase se référant à une méthode PCR non décrite dans le chapitre. Enfin, la Commission a ajouté le texte standard sur les témoins et l'extraction des acides nucléiques, ainsi que les tableaux répertoriant les amorces, sondes et paramètres des cycles pour la PCR en temps réel et pour la PCR conventionnelle, aux fins de la détection du virus de la nécrose hématopoïétique épizootique dans les tissus des poissons, tel que détaillé au point 7.4. de l'ordre du jour ci-après. L'inclusion des tableaux types a permis de supprimer certains détails des protocoles de PCR.

Dans la section 4.5. « Amplicon sequencing », le texte standard sur le séquençage de l'amplicon a remplacé le texte existant (voir le point 7.4. de l'ordre du jour ci-après).

Dans la section 5. « Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations », la Commission est convenue de préciser que les témoins testés au moyen d'une PCR en temps réel et pour lesquels un résultat positif a été obtenu devaient également être testés au moyen d'une PCR conventionnelle assortie d'une analyse séquentielle afin de distinguer le virus de la nécrose hématopoïétique épizootique des autres ranavirus.

Dans la section 6. « Corroborative diagnostic criteria », la Commission a procédé aux modifications génériques décrites au point 7 de l'ordre du jour ci-dessus.

Dans la section 6.1.1. « Definition of suspect case in apparently healthy animals », la Commission a remplacé le premier critère, « Positive result for EHNV based on virus isolation in cell cultures », par « EHNV-typical CPE in cell culture », qui est plus en adéquation avec le contenu du tableau 4.1.

La version révisée du chapitre 2.3.2. « Infection par le virus de la nécrose hématopoïétique épizootique » est présentée en annexe 26 afin que les Membres formulent leurs commentaires.

7.2.3. Section 2.2.1. du chapitre 2.3.9. « Infection par le virus de la virémie printanière de la carpe »

Réunion de septembre 2022

La Commission des animaux aquatiques est convenue d'inclure *Percocypris pingi* dans la liste des espèces sensibles figurant dans la section 2.2.1. du chapitre 2.3.9. « Infection par le virus de la virémie printanière de la carpe » (voir point 5.5.).

L'évaluation du groupe *ad hoc* sur la sensibilité de *Percocypris pingi* à l'infection par le virus de la virémie printanière de la carpe est présentée aux Membres en Annexe 10 à titre informatif.

La version révisée de la section 2.2.1. du chapitre 2.3.9. « Infection par le virus de la virémie printanière de la carpe » est présentée en annexe 27 afin que les Membres formulent leurs commentaires.

7.3. Section 2.4. « Maladies des mollusques »

7.3.1. Sections 2.2.1. et 2.2.2. du chapitre 2.4.2. « Infection à *Bonamia exitiosa* » et sections 2.2.1. et 2.2.2. du chapitre 2.4.3. « Infection à *Bonamia ostreae* »

Réunion de septembre 2022

La Commission a amendé les sections 2.2.1. et 2.2.2. du chapitre 2.4.2. « Infection à *Bonamia exitiosa* », et du chapitre 2.4.3. « Infection à *Bonamia ostrae* » afin qu'ils soient en ligne avec la taxonomie de *Magallana* (syn. *Crassotrea*) *ariakensis* et de l'huitre creuse du Pacifique (voir point 5.8.).

La version révisée des sections 2.2.1. et 2.2.2. du chapitre 2.4.2. « Infection à *Bonamia exitiosa* » est présentée aux Membres en annexe 28 afin qu'ils formulent leurs commentaires.

La version révisée des sections 2.2.1. et 2.2.2. du chapitre 2.4.3. « Infection à *Bonamia ostrae* » est présentée aux Membres en annexe 29 afin qu'ils formulent leurs commentaires.

7.3.2. Sections 2.2.1. et 2.2.2. du chapitre 2.4.4. « Infection à *Marteilia refringens* »

Réunion de septembre 2022

La Commission des animaux aquatiques a amendé les sections 2.2.1. et 2.2.2. du chapitre 2.4.4. « Infection à *Marteilia refringens* » en ligne avec les recommandations du groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE (voir point 5.8.)

La Commission n'a pas accepté la recommandation du groupe *ad hoc* d'inclure une espèce de copépode (*Paracartia grani*) dans l'article 11.4.2. (voir point 5.8.) Toutefois, la Commission a décidé d'ajouter le nouveau paragraphe suivant dans la section 2.2.1., afin que soit pris en compte le cas particulier où le risque est associé à un hôte intermédiaire : « Additionally, a copepod species (*Paracartia grani*) has been found to meet the criteria for listing as susceptible to infection with *Marteilia refringens* and is considered an intermediate host ». La Commission a estimé que la prise en compte de la sensibilité de *Paracartia grani* par les Membres pouvait s'avérer pertinente dans certaines situations afin de prévenir la propagation de *Marteilia refringens*.

La version révisée des sections 2.2.1. et 2.2.2. du chapitre 2.4.4. « Infection à *Marteilia refringens* » est présentée aux Membres en annexe 30 afin qu'ils formulent leurs commentaires.

8. Textes soumis aux Membres pour information

8.1. Proposition de tableau récapitulant les paramètres de réalisation de PCR en vue de l'harmonisation des protocoles des PCR

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a indiqué que le processus d'examen des chapitres mis à jour et reformatés avait permis de mettre en évidence la grande variabilité, d'un chapitre à l'autre, du niveau de détails fournis dans la description des méthodes reposant sur une technique PCR (polymerase chain reaction) figurant dans la section 4.4. « Nucleic acid amplification » ainsi que dans la présentation de cette information. La Commission a décidé de traiter cette question en élaborant un modèle dédié à la description des méthodes PCR. Ce modèle de paragraphe inclura, d'une part, un texte générique, uniforme et concis sur les méthodes d'extraction de l'acide nucléique et les témoins utilisés lors du test et, d'autre part, un tableau répertoriant toutes les informations nécessaires sur les séquences de l'amorce et des sondes ainsi que les paramètres des cycles.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partie B : point 3.1., page 9).

Réunion de septembre 2022

La Commission a examiné et approuvé la proposition de tableau répertoriant les séquences de l'amorce et des sondes, les paramètres des cycles ainsi que le texte sur les témoins, l'extraction de l'acide nucléique et le séquençage de l'amplicon. Le tableau et le texte, présentés ci-dessous, seront insérés dans le modèle de chapitre et dans l'ensemble des chapitres en cours de révision. Ainsi, l'information essentielle sur les méthodes PCR sera présentée de façon uniforme dans l'ensemble des chapitres du *Manuel aquatique*. Le tableau remplacera les textes actuels sur les protocoles de réalisation des méthodes PCR.

***Primers, probes* (*sequence*) *and cycling parameters* (*examples only*)**

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters

Reference, GenBank Accession No., Product size [bp]*)			
X	Fwd: TGC-GTC-CTG-CGT-ATG-GCA-CC Rev: GGC-TGG-CAT-GCC-CGA-ATA-GCA Probe: GGC-TGG-CAT-GCC-CGA-ATA-GCA	400 nM 300 nM	50 cycles of: 95°C/15 sec and 58°C/60 sec

*For conventional PCR only.

1. Section 4.4. *Nucleic acid amplification*

   PCR assays should always be run with the controls specified in Section X.X *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.X.0 *General information* (diseases of crustaceans or fish, as appropriate) [Section 2.6 *Molecular methods* of Chapter 2.4.0 *General information* (diseases of molluscs)]. Each sample should be tested in duplicate.

2. Section 4.4. *Nucleic acid amplification*

   Extraction of nucleic acids

   Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

3. Section 4.5. *Amplicon sequencing*

   The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced and analysed and compared with published sequences.

8.2. Chapitre 2.3.7. « Infection par l'iridovirus de la daurade japonaise »

Des commentaires ont été formulés par la Chine (Rép. populaire de), les États-Unis d'Amérique, la Norvège, la Suisse, le Taipei chinois et l'UE.

Contexte

Lors de sa réunion de février 2022, la Commission des animaux aquatiques a examiné le chapitre 2.2.7. « Infection par l'iridovirus de la daurade japonaise », qui a été mis à jour par les experts du Laboratoire de référence et reformaté selon le nouveau modèle de chapitre. La version révisée a été présentée aux Membres pour avis dans la partie B du rapport de février 2022 de la Commission.

Précédents rapports de la Commission des animaux aquatiques traitant de ce point :

Rapport de février 2022 (partir B : point 3.1.2.3. page 13).

Réunion de septembre 2022

La Commission a examiné le rapport du Groupe *ad hoc* sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE concernant l'infection par l'iridovirus de la daurade japonaise. Aux fins de l'inclusion du virus de la nécrose infectieuse rénale et splénique dans la Liste des maladies, la Commission est convenue qu'il était nécessaire de procéder à des évaluations complémentaires, et notamment de ses trois génogroupes : l'iridovirus de la daurade japonaise (RSIV), le virus de la nécrose infectieuse rénale et splénique (ISKNV) et l'iridovirus du corps rougeâtre du turbot (TRBIV) (voir le point 5.1.). La Commission a décidé de reporter l'examen des commentaires reçus sur la proposition de modification du chapitre 2.3.7. Elle a souhaité pouvoir examiner au préalable les commentaires qui seraient lui adressés sur la proposition d'inclusion de l'infection par le virus de la nécrose infectieuse rénale et splénique dans la Liste des maladies.

8.3. Développement de solutions destinées aux Membres en vue d'accélérer le processus d'actualisation des méthodes de diagnostic dans le *Manuel aquatique*

La Commission a identifié deux situations dans lesquelles la diffusion rapide d'information nouvelle et importante sur les tests de diagnostic figurant dans le *Manuel aquatique* s'avérait nécessaire. La première correspond au signalement de problèmes liés à la performance d'un test qui a été adopté et inclus dans le *Manuel aquatique*. La Commission a décidé que, dans ce type de situation, une note de bas de page pourrait être insérée dans le chapitre afin de détailler la nature du problème rencontré et de fournir des instructions sur la façon de le gérer. Une note de bas de page n'ayant pas pour vocation de remplacer ou de modifier un texte adopté, elle pourrait être immédiatement

ajoutée dans le chapitre concerné. Cette situation s'est déjà produite antérieurement, lorsque des problèmes de spécificités de tests avaient été signalés. La Commission souhaiterait également demander que des conseils soient prodigués aux Laboratoires de référence et aux Points focaux animaux aquatiques.

La seconde situation correspond à l'ajout de nouveaux tests de diagnostic dans le *Manuel aquatique*. Actuellement, les tests doivent faire l'objet d'une publication dans un journal à comité de lecture et doivent, de préférence, obtenir un niveau 2 lorsqu'ils sont soumis au processus de validation de l'OMSA. La Commission a été informée des travaux de la Commission des normes biologiques sur l'élaboration d'un modèle des données de validation que les candidats désireux de faire inclure leurs tests de diagnostic dans le *Manuel terrestre* devraient fournir (voir point 5.2.2. de l'ordre du jour du rapport de la Commission des normes biologiques de septembre 2022). Un membre de la Commission des animaux aquatiques a été chargé d'examiner ce modèle et de formuler des commentaires en retour sur sa pertinence et son applicabilité au *Manuel aquatique* lors de la réunion de février 2023. Si l'exigence de publier les informations relatives aux tests dans la littérature scientifique demeure, il pourrait toutefois être envisagé, dans les situations urgentes, que les développeurs du test soumettent leurs données conformément au modèle de validation des données. Il s'agirait d'une mesure provisoire, qui permettrait au test d'être inclus dans le *Manuel aquatique* avant sa publication dans un journal à comité de lecture.

9. Groupes *ad hoc*

9.1. Groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE

Le groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE s'est réuni pendant le mois de juin 2022 afin d'achever les évaluations de la sensibilité des espèces de mollusques à l'infection à *Marteilia refringens* (voir points 5.8. et 7.3.2.)

La Commission a été informée que le groupe *ad hoc* envisageait de se réunir en novembre 2022 afin de faire progresser ses travaux d'évaluation de la sensibilité des espèces à l'infection à *Perkinsus marinus*.

Le rapport de juin 2022 du groupe *ad hoc* sensibilité des espèces de mollusques à une infection par une maladie listée par l'OIE est disponible sur le site internet de l'OMSA.

9.2. Groupe *ad hoc* sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE

Le groupe *ad hoc* sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE s'est réuni pendant le mois d'avril 2022 afin de procéder aux évaluations de la sensibilité des espèces de poissons à l'infection par l'iridovirus de la daurade japonaise.

La Commission des animaux aquatiques a été informée que le groupe *ad hoc* n'a pas été en mesure d'achever ses évaluations de la sensibilité des espèces à l'infection par l'iridovirus de la daurade japonaise en raison de la complexité de l'agent pathogène. La Commission a examiné et a formulé des commentaires en retour sur le rapport intermédiaire du groupe *ad hoc*, qui fait état de la partie des travaux achevée à ce jour. Le groupe *ad hoc* envisage de se réunir à nouveau en novembre 2022 afin de finaliser les évaluations de la sensibilité des espèces à l'infection par l'iridovirus de la daurade japonaise.

9.3. Groupe *ad hoc* sur les nouveaux projets de chapitres relatifs à la préparation aux situations d'urgence sanitaire et à la gestion des foyers de maladie

La Commission des animaux aquatiques a examiné les travaux du groupe *ad hoc* sur la préparation aux situations d'urgence sanitaire et à la gestion des foyers de maladie. La Commission a remercié les membres de ce groupe pour leurs travaux sur les projets de chapitres. Elle a considéré que les travaux accomplis étaient suffisants pour lui permettre de progresser sur les deux chapitres. La Commission continuera ses travaux sur les projets de chapitre 4.X. « Préparation aux situations d'urgence sanitaire » et 4.Y. « Gestion des foyers de maladie » qu'elle examinera de façon plus approfondie lors de sa réunion de février 2023.

10. Candidatures au statut de centre de référence de l'OMSA ou changements d'experts

10.1. Évaluation des candidatures au statut de Centre de référence de l'OMSA dans le domaine de la santé des animaux aquatiques ou changements d'experts

La Commission des animaux aquatiques a examiné les demandes de changement d'experts et a recommandé l'acceptation des candidatures suivantes :

Infection par la septicémie hémorragique virale

Dr Britt Bang Jensen succèdera au Dr Niels Jørgen Olesen, suite au départ à son départ à la retraite, à la Technical University of Denmark National Institute for Aquatic Resources, Lyngby, Denmark.

10.2. Recherche de candidats au statut de Laboratoire de référence de l'OMSA

La Commission des animaux aquatiques a souligné la nécessité de désigner des Laboratoires de référence de l'OMSA pour les maladies suivantes :

Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique)

Infection à *Batrachochytrium dendrobatidis*

Infection à *Batrachochytrium salamandrivorans*

Infection par le virus de la myonécrose infectieuse

Infection à *Perkinsus marinus*

Infection à *Perkinsus olseni*

Infection par le virus du tilapia lacustre

Infection à *Xenohaliotis californiensis*.

La Commission invite les Membres disposant de l'expertise requise pour ces maladies à lui soumettre leur candidature.

11. Autres activités

11.1. Enregistrement des kits de tests de diagnostic

La Commission des animaux aquatiques a fait un point sur la situation actuelle du Registre des kits de diagnostic de l'OMSA avec le concours du Secrétariat de l'OMSA pour l'enregistrement des kits de diagnostic (OMSA SRDK). À ce jour, 14 kits de tests de diagnostic figurent dans le Registre des kits de diagnostic de l'OMSA.

La Commission a examiné les deux demandes d'enregistrement en cours pour des kits de diagnostic destinés aux animaux aquatiques ainsi que les deux demandes de renouvellement d'enregistrement pour cinq ans.

Lors de cet examen, la Commission a demandé des clarifications sur la procédure mise en œuvre pour s'assurer de l'adéquation entre les tests de diagnostic enregistrés et les méthodes listées dans les *Manuels aquatiques* et *terrestres* de l'OMSA. Cette demande sera plus amplement discutée en interne au sein du Service Antibiorésistance et produits vétérinaires.

_____…/Annexes

Annexe 1. Point 2 – Ordre du jour adopté

COMMISSION DES NORMES SANITAIRES POUR LES ANIMAUX AQUATIQUES DE L'OMSA

Réunion en format hybride, du 14 au 21 septembre 2022

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1. Directrice générale adjointe de l'OMSA
2. Adoption de l'ordre du jour
3. Réunion avec la Directrice générale
4. Coopération avec la Commission des normes sanitaires pour les animaux terrestres
5. Plan de travail de la Commission des animaux aquatiques
- 5.1. Approche pour l'établissement des premiers sujets prioritaires
  - 5.1.1. Chapitre 4.3. « Application de la compartimentation »
  - 5.1.2. Nouveau chapitre 5.X. « Échanges commerciaux d'animaux aquatiques d'ornement »
  - 5.1.3. Nouveau chapitre 5.Y. « Échanges commerciaux de matériel génétique »

6. Stratégie pour la santé des animaux aquatiques
- 6.1. Rapport sur l'état d'avancement de la mise en œuvre de la Stratégie
  - 6.1.1. Mise à jour sur la mise en œuvre
  - 6.1.2. Observatoire de l'OMSA – Résultats de l'enquête
  - 6.1.3. Réseau des Centres de référence – Service des Sciences
  - 6.1.4. Mise à jour du plan de travail concernant la résistance aux agents antimicrobiens

LE *CODE AQUATIQUE*

7. Points présentés aux Membres pour commentaire
- 7.1. Définitions du Glossaire : « Autorité compétente », « Autorité vétérinaire » et « Services chargés de la santé des animaux aquatiques » – examen de leur usage dans le *Code aquatique*
- 7.2. Marchandises dénuées de risques – Articles X.X.3. des chapitres spécifiques aux maladies
- 7.3. Articles révisés 8.X.3. des chapitres spécifiques aux maladies des amphibiens
- 7.4. Articles 11.X.3. révisés des chapitres spécifiques aux maladies des mollusques
- 7.5. Article 9.3.1. du chapitre 9.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) »
- 7.6. Articles 9.4.1. et 9.4.2. du chapitre 9.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse »
- 7.7. Article 10.9.2. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe »
- 7.8. Nouveau chapitre 10.X. « Infection par le virus du tilapia lacustre »
- 7.9. Articles 11.4.1. et 11.4.2. du chapitre 11.4. « Infection à *Marteilia refringens* »
- 7.10. Harmonisation des chapitres spécifiques aux maladies des mollusques : Articles 11.X.8. – 11.X.12.

8. Points à aborder par la Commission des animaux aquatiques
- 8.1. Évaluation des périodes établies par défaut dans les Articles X.X.4. – X.X.8. des chapitres spécifiques aux maladies
- 8.2. État d'avancement des travaux menés sur les marchandises dénuées de risques
- 8.3. Examen de maladies émergentes
  - 8.3.1. Infection par le virus de l'oedème de la carpe (CEV)
  - 8.3.2. CMNV chez le poisson zèbre
  - 8.3.3. *Coxiella burnetii* chez les crevettes (sujet proposé par WAHIAD)
  - 8.3.4. Examen des propositions de procédures pour les maladies émergentes
- 8.4. Discussion sur la certification électronique
- 8.5. Consultants en stratégie de la faune sauvage

LE *MANUEL AQUATIQUE*

9. **Points destinés à recueillir les commentaires des Membres**
- 9.1. Section 2.2. « Maladies des crustacés »
  - 9.1.1. Chapitre 2.2.0. « Informations générales (Maladies des crustacés) »

9.1.2. Chapitre 2.2.1. « Maladie de nécrose hépatopancréatique aiguë »
9.1.3. Chapitre 2.2.2. « Infection à *Aphanomyces astaci* (peste de l'écrevisse) »
9.1.4. Chapitre 2.2.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) »
9.1.5. Chapitre 2.2.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse »
9.1.6. Chapitre 2.2.5. « Infection par le virus de la myonécrose infectieuse »
9.1.7. Chapitre 2.2.6. « Infection par le nodavirus de *Macrobrachium rosenbergii* (maladie des queues blanches) »
9.1.8. Chapitre 2.2.7. « Infection par le virus du syndrome de Taura »
9.1.9. Chapitre 2.2.8. « Infection par le virus du syndrome des points blancs »
9.1.10. Chapitre 2.2.9. « Infection par le génotype 1 du virus de la tête jaune »

9.2. Section 2.3. « Maladies des poissons »
9.2.1. Chapitre 2.3.1. « Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique) »
9.2.2. Chapitre 2.3.2. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse »
9.2.3. Chapitre 2.3.7. « Infection par l'iridovirus de la daurade japonaise »
9.2.4. Section 2.2.2. du chapitre 2.3.9. « Infection par le virus de la virémie printanière de la carpe »

9.3. Section 2.4. « Maladies des mollusques »
9.3.1. Sections 2.2.1. et 2.2.2. du chapitre 2.4.4. Infection à *Marteilia refringens*

10. Proposition de tableau récapitulant les paramètres de réalisation de PCR en vue de l'harmonisation des protocoles des PCR

11. Points à aborder par la Commission des animaux aquatiques

11.1. Développement de solutions destinées aux Membres en vue d'accélérer le processus d'actualisation des méthodes de diagnostic dans le *Manuel aquatique*

12. Groupes *ad hoc*

12.1. Rapport du Groupe *ad hoc* sur la sensibilité des espèces de mollusques à l'infection par une maladie listée par l'OIE

12.2. Projet de rapport du Groupe *ad hoc* sur la sensibilité des espèces de poissons à l'infection par une maladie listée par l'OIE

12.3. Projet de rapport du Groupe *ad hoc* sur les nouveaux projets de chapitres relatifs à la préparation aux situations d'urgence sanitaire et à la gestion des foyers de maladie

13. Centres de référence ou changement d'experts

Évaluation des candidatures au statut de Centre de référence de l'OMSA dans le domaine de la santé des animaux aquatiques ou changements d'experts

14. Sujets divers

14.1. Pour discussion
14.1.1. Enregistrement des kits de diagnostic
14.1.1.1. IQ PlusTM WSSV Kit
14.1.1.2. IQ 2000TM WSSV
14.1.1.3. WSSV LFT
14.1.1.4. Genic Shrimp Multipath testing package
14.1.2. Procédures d'auto-déclaration d'un statut indemne de maladie
14.1.3. Rapport de février – Parties A et B

14.2. Pour information
14.2.1. Coordination de la recherche à l'OIE
14.2.2. L'impact mondial des maladies animales (GBADs)

15. Revue de la réunion

16. Prochaine réunion : 15– 22 février 2023

Annexe 2. Point 2 – Liste des participants

COMMISSION DES NORMES SANITAIRES POUR LES ANIMAUX AQUATIQUES DE L'OMSA

Réunion en format hybride, du 14 au 21 septembre 2022

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MEMBRES DE LA COMMISSION

Dr Ingo Ernst
(Président)
Director Aquatic Pest and Health Policy,
Department of Agriculture, Water and the Environment,
Canberra,
AUSTRALIE

Dr Alicia Gallardo Lagno
(Vice-président)
Senior advisor FARMAVET,
University of Chile,
La Pintana,
CHILI

Dr Prof. Hong Liu
(Membre)
Deputy Director,
Animal and Plant Inspection and Quarantine Technical Centre,
Shenzhen City,
CHINE (République populaire de)

Dr Fiona Geoghegan
(Vice-président)
Legislative Officer,
European Commission,
DG SANTE
Brussels,
BELGIQUE

Dr Kevin William Christison
(Member)
Specialist Scientist,
Department of Forestry, Fisheries and the Environment,
Vlaeberg,
AFRIQUE DU SUD

Dr Espen Rimstad
(Member)
Professor in Virology,
Norwegian University of Life Sciences
Ås,
NORVÈGE

AUTRES PARTICIPANTS

Dr Mark Crane
CSIRO Honorary Fellow,
Research Group Leader | AAHL Fish Diseases Laboratory
Australian Centre for Disease Preparedness (ACDP) I CSIRO,
Geelong,
AUSTRALIE

SIÈGE DE L'OMSA

Dr Gillian Mylrea
Cheffe
Service des Normes

Dr Stian Johnsen
Chargé de mission
Service des Normes

Ms Sara Linnane
Agent scientifique – Normes internationales
Service des Sciences

Dr Bernita Giffin
Coordinatrice scientifique pour la santé des animaux aquatiques
Service des Normes

Dr Gounalan Pavade
Coordinateur scientifique
Service des Sciences

Annexe 3. Point 4. – Plan de travail et priorités

PLAN DE TRAVAIL DE LA COMMISSION DES ANIMAUX AQUATIQUES

Travaux en cours en vue d'une adoption en 2023 ou à une date ultérieure

Code aquatique			
Chapitre/Sujet	**Statut**		
	Septembre 2022	**Février 2023**	**Session générale de mai 2023**
Suivi des maladies émergentes et examen des actions à prendre	En cours		
Définitions du Glossaire : « Autorité compétente », « Autorité vétérinaire » ''et « Services chargés de la santé des animaux aquatiques »		Examen de l'usage dans le *Code aquatique* et présentation des amendements pour commentaire	
Chapitre 1.3. Maladies listées par l'OIE – Inclusion de l'infection par le virus de la nécrose infectieuse rénale et splénique dans la Liste des maladies de l'OIEe	Nouvelle évaluation en vue d'une inclusion dans la liste et présentation des amendements pour commentaire	Examen des commentaires des Membres	
Procédures officielles pour l'auto-déclaration de statut indemne		Rédaction d'un modèle d'auto-déclaration afin d'accompagner les Membres lors de la soumission de leurs auto-déclarations	
Chapitre 4.3. « Application de la compartimentation »	Questionnaire destiné aux Membres	Examen des réponses fournies par les Membres sous-tendant toute démarche d'amendement du chapitre 4.3.	
Chapitre 4.X. Nouveau projet de chapitre sur la préparation aux situations d'urgence		Révision du projet de chapitre 4.X.	
Chapitre 4.Y. Nouveau projet de chapitre sur la gestion des foyers de maladie		Révision du projet de chapitre 4.Y.	
Chapitre 5.2. « Procédures de certification »		Discussion du plan de modifications	
Chapitres 5.6. à 5.9.		Revue du rapport du Groupe *ad hoc* et de la Commission du Code	
Chapitre 5.X. « Commerce d'animaux aquatiques d'ornement »	Élaboration d'un plan pour la rédaction du nouveau chapitre	Révision de l'ébauche d'un premier chapitre	
Chapitre 5.Y. « Commerce de matériels génétiques »	Élaboration d'un plan pour la rédaction du nouveau chapitre	Révision de l'ébauche d'un premier chapitre	
Marchandises dénuées de risques – Chapitres spécifiques aux maladies – Articles X.X.3.		Examen des évaluations mises à jour des marchandises dénuées de risques et des articles amendés. Soumission de la version amendée des articles pour avis	
Évaluation des périodes établies par défaut dans les articles X.X.4. – X.X.8. des chapitres spécifiques aux maladies	Établissement d'une méthode pour évaluer les périodes établies par défaut	Soumission de l'évaluation des périodes établies par défaut pour avis	

Espèces sensibles – Maladies des crustacés – Articles 9.X.1. et 9.X.2. pour : – l'infection par le virus iridescent des décapodes – l'infection à *Aphanomyces astaci* (peste de l'écrevisse)		Convocation d'un groupe *ad hoc*	
Article 9.3.1. du chapitre 9.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante)	Examen des commentaires (premier cycle de consultation)	Examen des commentaires (second cycle de consultation)	Projet de texte proposé à l'adoption
Articles 9.4.1. et 9.4.2. du chapitre 9.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse »	Examen des commentaires (premier cycle de consultation)	Examen des commentaires (second cycle de consultation)	Projet de texte proposé à l'adoption
Article 9.4.2. du chapitre 9.5. « Infection par le virus de la myonécrose infectieuse »	Examen de la version amendée de l'article et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Espèces sensibles – Maladies des poissons – Articles 10.X.1. et 10X.2. pour : – l'infection par l'iridovirus de la daurade japonaise – l'infection par le virus du tilapia lacustre – l'infection à *Aphanomyces astaci* (syndrome ulcératif épizootique)	Revue du projet de rapport du groupe *ad hoc*. Prochaine réunion du groupe *ad hoc* prévue en novembre 2022	Révision de la version amendée des articles sur l'infection par l'iridovirus de la daurade japonaise.	
Article 10.9.2. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe »	Revue de l'évaluation réalisée par un groupe *ad hoc* et de la version amendée de l'article et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Espèces sensibles Évaluation de nouvelles espèces ou de nouveaux éléments probants portant sur des maladies précédemment évaluées si besoin	En cours		
Chapitre 10.X. « Infection par le virus du tilapia lacustre »	Examen du projet de chapitre et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Espèces sensibles – Maladies des mollusques – Articles 11X.1. et 11X.2. pour : – l'infection à *Marteilia refringens* – l'infection à *Perkinsus marinus* – l'infection à *Xenohaliotis californiensis* – l'infection à *Perkinsus olseni*	*Marteilia refringens* : examen de la version amendée des articles et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
	Prochaine réunion du groupe *ad hoc* prévue en novembre 2022	*Perkinsus marinus* : examen de la version amendée des articles et soumission pour avis	
Espèces sensibles – Article 11.2.2. du chapitre 11.2. « Infection à *Bonamia exitiosa* »	Examen de la version amendée de l'article et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Espèces sensibles – Article 11.3.2. du chapitre 11.3. « Infection à *Bonamia ostrea* »	Examen de la version amendée de l'article et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Modèles d'articles 11.X.9. – 11.X.12. : harmonisation avec d'autres chapitres spécifiques aux maladies	Examen de la version amendée des articles et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Manuel aquatique			
Chapitre/Sujet	**Statut**		
	Septembre 2022	**Février 2023**	**Session générale de mai 2023**
Section 2.2. « Dispositions générales – Crustacés	Examen de la version amendée du chapitre et soumission pour avis	Examen des commentaires (premier cycle de consultation)	
Chapitre 2.1.1. « Maladie de nécrose hépatopancréatique aiguë »	Examen des commentaires (premier cycle de consultation)	Examen des commentaires (deuxième cycle de consultation)	Projet de texte proposé à l'adoption
Chapitre 2.2.2. « Infection à *Aphanomyces astaci* (peste de l'écrevisse) »	Nouvel examen de la version mise à jour du projet de texte et soumission pour avis	Examen des commentaires (premier cycle de consultation)	

Chapitre 2.2.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) »	Examen des commentaires (premier cycle de consultation)	Examen des commentaires (deuxième cycle de consultation)	Projet de texte proposé à l'adoption
Chapitre 2.2.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuses »	Examen des commentaires (premier cycle de consultation)	Examen des commentaires (deuxième cycle de consultation)	Projet de texte proposé à l'adoption
Chapitre 2.2.5. « Infection par le virus de la myonécrose infectieuse »	Mise à jour, reformatage puis soumission de la nouvelle version aux Membres pour avis	Examen des commentaires (premier cycle de consultation)	
Chapitre 2.2.6. « Infection par le nodavirus de *Macrobrachium rosenbergii* (maladie des queues blanches)	Examen de la version mise à jour du projet de texte	Nouvel examen de la version mise à jour du projet de texte, et soumission pour avis	
Chapitre 2.2.7. « Infection par le virus du syndrome de Taura »	Mise à jour, reformatage puis soumission de la nouvelle version aux Membres pour avis	Examen des commentaires (premier cycle de consultation)	
Chapitre 2.2.8. « Infection par le virus du syndrome des points blancs »	Mise à jour, reformatage puis soumission de la nouvelle version aux Membres pour avis	Examen des commentaires (premier cycle de consultation)	
Chapitre 2.2.9. « Infection par le génotype 1 du virus de la tête jaune »	Mise à jour, reformatage puis réexamen	Nouvel examen de la version mise à jour du projet de texte, et soumission pour avis	
Chapitre 2.2.X. « Infection par le virus 1 iridescent des décapodes »		Élaboration d'un projet de chapitre destiné à être révisé	
Chapitre 2.3.1. « Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique) »	Examen des commentaires (premier cycle de consultation)	Examen des commentaires (second cycle de consultation)	Projet de texte proposé à l'adoption
Chapitre 2.3.2. « Infection par le virus de la nécrose hématopoïétique épizootique »	Examen des commentaires (second cycle de consultation)	Examen des commentaires (troisième cycle de consultation)	Projet de texte proposé à l'adoption
Chapitre 2.3.7. « Infection par l'iridovirus de la daurade japonaise »		Examen du rapport du groupe *ad hoc* et révision selon l'approche retenue pour l'inclusion dans la liste de l'OIE	
Section 2.2.1. du chapitre 2.3.9. « Infection par le virus de la virémie printanière de la carpe »	Examen de la version amendée des articles et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Chapitre 2.3.X. « Infection par le virus du tilapia lacustre »		Élaboration d'un projet de chapitre à réviser	
Sections 2.2.1. et 2.2.2. du chapitre 2.4.4. « Infection à *Marteilia refringens* »	Examen de la version amendée des articles et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Sections 2.2.1. et 2.2.2. du chapitre 2.4.2. « Infection à *Bonamia exitiosa* »	Examen de la version amendée des articles et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption
Sections 2.2.1. et 2.2.2. du chapitre 2.4.3. « Infection à *Bonamia ostreae* »	Examen de la version amendée des articles et soumission pour avis	Examen des commentaires (premier cycle de consultation)	Projet de texte proposé à l'adoption

Hiérarchisation des tâches à entreprendre avant mai 2024 selon leur degré de priorité

Code aquatique				
Chapitre/Sujet	**Statut**	**Degré de priorité 1**	**Degré de priorité 2**	**Prochaines étapes**
Chapitre 1.3. « Maladies listées par l'OIE »	Examen des maladies nouvelles ou non en vue de leur inclusion ou de leur retrait de la Liste des maladies de l'OIE	En cours		

Chapitre 4.2. « Zonage et compartimentation »	Modification du chapitre afin de l'axer uniquement sur le zonage		✓	
Manuel aquatique				
Chapitre/Sujet	**Statut**	**Degré de priorité 1**	**Degré de priorité 2**	
Chapitre 2.4.0. « Informations générales » du Titre 2.4. « Maladies des mollusques »	Examen et mise à jour du chapitre introductif sur les maladies des mollusques		✓	
Chapitres 2.4.X. « Chapitres spécifiques aux maladies des mollusques »	Mise à jour et reformatage des chapitres selon le nouveau modèle de chapitre (pour toutes les maladies)		✓	

Annexe 4. Point 5.1. – Chapitre 1.3. « Maladies listées par l'OIE » – Inclusion de l'infection par des *Megalocytivirus*

CHAPITRE 1.3.

MALADIES LISTÉES PAR L'OIE

[…]

Article 1.3.1.

Sont listées par l'OIE, dans la catégorie des *maladies* des poissons, les *maladies* suivantes :

– Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique)

– Infection à *Gyrodactylus salaris*

– Infection par des variants délétés dans la RHP du virus de l'anémie infectieuse du saumon ou par des variants RHP0 de ce virus

– Infection par l'alphavirus des salmonidés

– Infection par l'herpèsvirus de la carpe koï

– ~~Infection par l'iridovirus de la daurade japonaise~~

– Infection par le virus de la nécrose hématopoïétique épizootique

– Infection par le virus de la nécrose hématopoïétique infectieuse

- Infection par le *virus de la nécrose infectieuse rénale et splénique*

– Infection par le virus de la septicémie hémorragique virale

– Infection par le virus de la virémie printanière de la carpe

– Infection par le virus du tilapia lacustre.

[…]

Annexe 5. Point 5.1. – Chapitre 1.3. « Maladies listées par l'OIE » – Inclusion de l'infection par des *Megalocytivirus*

ÉVALUATION DE L'INFECTION PAR LE VIRUS DE LA NÉCROSE INFECTIEUSE RÉNALE ET SPLÉNIQUE EN VUE DE SON INCLUSION DANS LA LISTE DES MALADIES DU *CODE SANITAIRE POUR LES ANIMAUX AQUATIQUES* DE L'OMSA

Récapitulatif de l'évaluation

1. La Commission des normes sanitaires pour les animaux aquatiques a évalué l'infection par le virus de la nécrose infectieuse rénale et splénique, et notamment ses trois génogroupes que sont l'iridovirus de la daurade japonaise (RSIV), le virus de la nécrose infectieuse rénale et splénique (ISKNV) et l'iridovirus du corps rougeâtre du turbot (TRBIV) au regard des critères d'inclusion dans la liste des maladies figurant à l'article 1.2.2. du *Code aquatique*.

2. La Commission des animaux aquatiques est convenue que le génogroupe RSIV, actuellement listé dans le *Code aquatique*, ainsi que les deux génogroupes ISKNV et TRIBV satisfaisaient aux critères 1, 2, 3, et 4b (voir Tableau 1 ci-dessous).

3. La Commission des animaux aquatiques a noté que les trois génogroupes présentaient des similitudes en matière d'espèces sensibles, d'épidémiologie et de méthodes de diagnostic. À ce titre, la Commission a estimé que la maladie devait être listée sous la désignation « Infection par le virus de la nécrose infectieuse rénale et splénique (ISKNV) ». Il est proposé que la définition de l'infection par le virus de la nécrose infectieuse rénale et splénique désigne une infection causée par les trois génogroupes (ISKNV, RSIV and TRBIV) mais exclue une autre espèce de *Megalocytivirus*, le virus de la maladie de perte d'écailles (« scale drop disease virus »).

	Critères d'inclusion dans la Liste de l'OIE						Conclusion
	1	2	3	4a	4b	4c	
Infection par l'ISKNV	+	+	+	NA	+	-	La maladie satisfaisait aux critères d'inclusion dans la Liste de l'OIE.

NA = non applicable.

Critères d'inclusion figurant au chapitre 1.2. du *Code aquatique*

Les critères d'inclusion d'une maladie dans la liste de l'OIE sont les suivants :

1. La propagation internationale de l'agent pathogène (via des animaux aquatiques, des produits issus d'animaux aquatiques, des vecteurs ou des matériels contaminés) est probable.

ET

2. Au moins un pays peut démontrer l'absence de la maladie sur son territoire ou dans une zone chez les animaux aquatiques sensibles, conformément aux dispositions prévues au chapitre 1.4.

ET

3. Une définition de cas précise est disponible et il existe une méthode fiable de détection et de diagnostic.

ET

4.a. La transmission naturelle à l'homme a été prouvée, et la présence de l'infection chez l'homme est associée à des conséquences graves.

OU

4.b. Lorsqu'elle apparaît, il est prouvé que la maladie affecte la santé des animaux aquatiques d'élevage à l'échelle d'un pays ou d'une zone, avec de lourdes conséquences telles que, par exemple, des pertes de production, une morbidité ou une mortalité constatées au niveau du pays ou de la zone.

OU

4.c. On a montré la présence de la maladie ou on dispose d'éléments de preuve scientifiques indiquant que la maladie affecterait la santé des animaux aquatiques sauvages avec de lourdes conséquences telles que, par exemple, une morbidité ou une mortalité à l'échelle de la population, une baisse de productivité ou des répercussions sur l'écologie.

Contexte

Le genre *Megalocytivirus* est un des sept genres de la famille des *Iridiviridae*. À l'instar des genres *Ranavirus* et *Lymphocystivirus*, il appartient à la sous-famille des Alphairidovirinae (Chinchar *et al*., 2017 ; Chinchar *et al*., 2020). Les mégalocytivirus se différencient des ranavirus et des lymphocystivirus par leur capacité à induire une augmentation marquée de la taille des cellules des tissus infectés et par l'analyse séquentielle des gènes viraux principaux (Chinchar *et al*., 2017). Les mégalocytivirus sont les agents étiologiques de maladies graves associées à des mortalités importantes chez plusieurs espèces de poissons marins et dulçaquicoles (Kurita & Nakajima, 2012).

Le Comité international sur la taxonomie des virus (ICTV) reconnait deux espèces appartenant au genre *Megalocytivirus* : le virus de la nécrose infectieuse rénale et splénique (ISKNV) et le virus de la maladie de perte d'écailles (SDDV) (Chinchar *et al*., 2017). Le SDDV se distingue d'un point de vue génétique et épidémiologique de l'ISKNV et donc n'est pas inclus dans la présenté évaluation.

Au sein de l'espèce ISKNV ont été reconnus trois génogroupes : l'ISKNV, le RSIV et le TRBIV (Song *et al*., 2008). Toutefois, il n' pas encore été déterminé si ces trois génogroupes correspondaient à des espèces distinctes ou à des souches distinctes d'une seule et même espèce (Chinchar *et al*., 2017). Les mégalocytivirus portent des noms variés et choisis d'après l'espèce dans laquelle ils ont été détectés pour la première fois ; toutefois, tous les variants de l'espèce ISKNV dont le génome a été analysé ont été placés dans un des trois génogroupes (ISKNV, RSIV and TRBIV) (Chinchar *et al*., 2017).

Le nom « ISKNV » est employé pour désigner une des deux espèces reconnues du genre *Megalocytivirus* mais également pour désigner un des trois génogroupes de cette espèce. Dans le présent document, l'emploi du terme « ISKNV » fait référence au génogroupe ISKNV alors que le terme « espèce ISKNV » fait référence à l'espèce.

L'infection par l'iridovirus de la daurade japonaise a été listée dans le *Code sanitaire* [1] *pour les animaux aquatiques* pour la première fois en 2003. Son inclusion dans la liste a été maintenue depuis et il figure dans l'édition de 2022 du *Code aquatique*. La maladie causée par le RSIV a été détectée pour la première fois dans un élevage de dorades roses (*Pagrus major*), au Japon, en 1990 (Inouye *et al*., 1992). Le RSIV a été principalement détecté chez des poissons marins. Parmi les espèces listées comme étant sensibles [2] à l'infection par le RSIV dans le *Code aquatique* de l'OMSA figurent dorades roses (*Pagrus major*), la sériole du Japon (*Seriola quinqueradiata*), la sériole couronnée (*Seriola dumerili*), une espèce de *Lateolabrax*, la perche barramundi (*Lates calcarifer*), le thon rouge de l'Atlantique (*Thunnus thynnus*), *Oplegnathus fasciatus*, la carangue dentue (*Caranx delicatissimus*), *Siniperca chuatsi*, le tambour rouge (*Sciaenops ocellatus*), le mulet à grosse tête (*Mugil cephalus*) et des espèces de mérous (*Epinephelus spp.*).

Le génogroupe ISKNV n'est actuellement pas listé dans le *Code aquatique* de l'OMSA. La morphologie des virions est évocatrice de celle des iridovirus. En outre, l'augmentation de la taille des cellules présentant des corps d'inclusion similaires à ceux des mégalocytivirus a été rapportée chez des espèces de poissons dulçaquicoles depuis la fin des années 80 et des années 90 (par exemple, Armstrong & Ferguson, 1989 ; Anderson *et al*., 1993). L'ISKNV a été détecté dans des échantillons de poissons d'ornements provenant d'archives datant de 1996 (Go *et al*., 2006 ; Go *et al*., 2016). L'infection par le virus de la nécrose infectieuse rénale et splénique a été décrite chez *Siniperca chuatsi*; (He *et al*., 2000 ; He *et al*., 2002). En 2001, le génome de l'ISKNV a été analysé et il a été conclu qu'il était similaire à celui du RSIV (He *et al*., 2001). L'ISKNV a été détecté chez de nombreux poissons dulçaquicoles, notamment de nombreuses espèces de la filière des poissons d'ornement (voir la revue de Johan & Zainathan, 2020). La présence de ce génotype a été rapportée chez de nombreuses espèces de poissons d'ornement faisant l'objet d'échanges commerciaux au niveau international. Il a été également rapporté que l'ISKNV était responsables de mortalités massives d'espèces importantes pour la consommation humaine (par exemple, Subramaniam *et al*., 2016 ; Ramírez-Paredes *et al*., 2020).

Le génogroupe de l'iridovirus du corps rougeâtre du turbot (TRBIV) n'est actuellement pas inclus dans le *Code aquatique* de l'OMSA. Il a été décrit pour la première fois comme responsable de la maladie chez le turbot *Scophthalmus maximus* (Shi *et al.,* 2004). Le TRBIV est connu comme la cause principale de maladie chez les poissons plats en Chine et en Corée (par exemple Shi *et al.,* 2004 ; Do *et al.,* 2005). En outre, il a également été détectée chez d'autres espèces, notamment celles de la filière de poissons d'ornement (Go *et al*., 2016 ; Koda *et al*., 2018). Le TRBIV est également à l'origine de maladie chez d'autres espèces d'élevage présentant une importance économique telles que la perche barramundi (*Lates calcarifer*) (Tsai *et al*., 2020) et *Oplegnathus fasciatus* (Huang *et al*., 2011).

[1] Le RSIV a été inclus dans le *Code aquatique* avant 2003, mais comme « autre maladie d'importance ».
[2] Il faut noter que l'évaluation des espèces listées comme étant sensibles à l'infection par le RSIV conformément au chapitre 1.5. du *Code aquatique* n'a pas été achevée.

La Commission des animaux aquatiques a précédemment proposé une approche pour différencier les souches d'agents pathogènes (se référer aux rapports de la Commission de février et octobre 2011). Les trois principaux critères qui ont été pris en compte pour l'applicabilité de la différenciation des souches de l'agent pathogène dans les normes du *Code aquatique* et du *Manuel aquatique* étaient les suivants : 1) les variants de l'agent pathogène sont clairement identifiées dans la littérature scientifique et les caractéristiques des maladies qu'ils causent sont différentes ; 2) il existe des méthodes robustes qui permettent de toujours les différencier ; 3) il y a ou il peut possiblement y avoir une gestion différenciée des variants dans ou entre les pays. Dans le cas de l'espèce ISKNV, le RSIV a été listé avant même que soit conduites les travaux de recherche pour définir ses trois génogroupes ainsi que pour établir leurs liens épidémiologiques et génétiques. Parce que la Liste inclut le RSIV et non l'ISKNV ou le TRBIV, la présente évaluation expose les informations spécifiques à chacun de ces trois génogroupes. Toutefois, il est proposé de lister les trois génogroupes comme étant l'espèce ISKNV.

Évaluation au regard des critères d'inclusion dans la Liste

Critère n°1. La propagation internationale de l'agent pathogène (via des animaux aquatiques, des produits issus d'animaux aquatiques, des vecteurs ou des matériels contaminés) est probable.

Évaluation

L'espèce ISKNV peut être transmise horizontalement par l'eau et demeure viable dans les tissus congelés de l'hôte. Il est attendu que la probabilité de transmission soit plus grande dans la filière de commercialisation des poissons vivants mais elle est également possible dans les produits issus d'animaux aquatiques, en particulier si ces derniers ne sont pas éviscérés.

De nombreux poissons marins et dulçaquicoles sont sensibles à l'espèce ISKNV. Ils font l'objet d'échanges commerciaux au niveau international, que ce soit sous forme de poissons vivants (destinés à la consommation humaine, à l'aquaculture ou un usage ornemental) ou de produits issus d'animaux aquatiques.

Le RSIV a été détecté dans plusieurs pays d'Asie où il a été associé à des foyers de maladies chez des espèces de poissons d'élevage marins (Kurita & Nakajima, 2012). Certaines espèces, destinées à la consommation humaine, sont commercialisées vivantes (par exemple la dorade rose, les mérous), alors que d'autres sont commercialisées sous forme de produits issus d'animaux aquatiques.

L'ISKNV a été détecté chez de nombreuses espèces commercialisées dans la filière des poissons d'ornement. Cette filière a été mise en cause dans l'apparition et la propagation de la maladie (par exemple, Jeong *et al*., 2008 ; Johan & Zainathan, 2020). Les poissons d'ornement infectés peuvent ne pas présenter de signes cliniques (par exemple, Subramaniam et al., 2014) et, à ce titre, peuvent avoir un rôle de porteurs du virus. L'ISKNV a également été détecté chez des espèces d'élevage d'importance pour la consommation humaine, et qui font l'objet d'échanges commerciaux au niveau international, telles que le tilapia (Ramírez-Paredes *et al*., 2020). L'ISKNV a également été détecté chez des poissons non transformés utilisés comme aliment pour les animaux d'aquaculture (Lajimin *et al*., 2015), ce qui suggère que les poissons commercialisés comme aliment pour animaux en aquaculture ou comme appâts pourraient constituer une voie d'introduction. La transmission de l'agent pathogène aux espèces de poissons marins par des espèces de poissons dulçaquicoles a été démontrée par inoculation directe et cohabitation (Jeong *et al*., 2008b ; Go & Whittington, 2019).

Le TRBIV a été observé chez plusieurs espèces d'importance pour les échanges internationaux (par exemple, le turbot, le cardeau hirame, la perche barramundi), qu'elles soient commercialisées vivantes ou sous forme de produits issus d'animaux aquatiques. Les résultats de l'analyse phylogénétique indiquent une récente propagation internationale du TRBIV (Tsai *et al*., 2020).

Les variants de l'espèce ISKNV ont été détectés chez de nombreuses espèces d'espèces de poissons marins et dulçaquicoles qui font l'objet d'échanges internationaux. Chacun des trois génogroupes a été détecté dans des marchandises commercialisées et il y a des preuves établissant un lien de causalité entre la propagation internationale et les échanges commerciaux.

Conclusion

Le critère est satisfait.

Critère n°2. Au moins un pays peut démontrer l'absence de la maladie sur son territoire ou dans une zone chez les animaux aquatiques sensibles conformément aux dispositions prévues au chapitre 1.4.

Évaluation

L'infection par le RSIV est notifiable à l'OMSA depuis 2003. Plusieurs pays continuent de rapporter qu'ils n'ont jamais détecté le virus sur leur territoire (se référer au Système Mondial d'information Zoosanitaire de l'OMSA) ; il est probable que certains de ces pays puissent démontrer leur statut indemne de la maladie.

La présence de l'ISKNV a été rapportée chez un grand nombre de poissons commercialisés dans la filière des poissons d'ornement et il est fortement probable que ce génogroupe soit largement répandu le long des chaînes d'approvisionnement. Toutefois, certains pays réunissent de façon continue des conditions élémentaires de sécurité biologique [3] pour l'ISKNV et sont en capacité de démontrer leur statut indemne. En outre, les tests PCR utilisés aux fins de la surveillance du RSIV détecteraient également l'ISKNV, ce qui constituerait la preuve de l'absence d'ISKNV.

Le TRBIV a été détecté pour la première fois dans des élevages de poissons plats en Chine et en Corée. Il a également été détecté chez des poissons d'ornement et dans les élevages de perches barramundi. Les tests PCR recommandés dans le chapitre du *Manuel aquatique* dédié à l'infection par le RSIV pourraient ne pas détecter le TRIBV, avec comme conséquence une moins grande confiance concernant la distribution de ce génogroupe. Toutefois, le TRBIV ayant été démontré comme étant pathogène pour les populations de certaines espèces d'élevage, il est probable qu'il soit détecté en cas d'apparition de la maladie chez ces espèces. Bien qu'il y ait moins de certitude concernant la distribution du TRBIV, il semble probable qu'au moins un pays pourrait déposer une auto-déclaration d'absence pour l'intégralité du pays ou une zone.

Conclusion

Le critère est satisfait.

Critère n°3. Une définition de cas précise est disponible et il existe une méthode fiable de détection et de diagnostic.

Évaluation

Les définitions de cas pour la suspicion et la confirmation de l'infection par le RSIV sont disponibles dans le *Manuel aquatique* de l'OMSA. Étant donné que la plupart des tests PCR pour la RSIV (et d'autres méthodes comme, par exemple, l'histologie) détectent l'ISKNV, les définitions de cas pourraient être aisément adaptées afin d'inclure l'ISKNV. Kawato *et al.* (2021) ont comparé les performances analytiques de quatre méthodes de PCR en temps réel pour la détection des mégalocytivirus (à l'exclusion du SDDV). Ils ont démontré que trois des quatre tests détectaient les virus ciblés, à savoir l'ISKNV, le RSIV, et le TRBIV. Le nombre d'outils de diagnostic disponibles pour détecter les espèces ISKNV et élaborer les définitions de cas incluant les trois génogroupes est suffisant.

Conclusion

Le critère est satisfait.

Critère n°4.a. La transmission naturelle à l'homme a été prouvée, et la présence de l'infection chez l'homme est associée à des conséquences graves.

Évaluation

Il n'y a aucune preuve de transmission à 'homme.

Conclusion

Le critère n'est pas applicable.

Critère n°4b. Lorsqu'elle apparaît, il est prouvé que la maladie affecte la santé des animaux aquatiques d'élevage à l'échelle d'un pays ou d'une zone, avec de lourdes conséquences telles que, par exemple, des pertes de production, une morbidité ou une mortalité constatée au niveau du pays ou de la zone.

Évaluation

Le RSIV a causé des mortalités massives dans les populations de poissons d'élevage. La maladie a été détectée pour la première fois chez la dorade rose au Japon et se caractérisait par une léthargie, une anémie sévère, des pétéchies au niveau des branchies et une splénomégalie (Inouye *et al*., 1992 ; Jung *et al*., 1997 ; Nakajima & Maeno, 1998). Il a été

[3]Les conditions élémentaires de sécurité biologique sont définies dans l'article 1.4.6. du *Code aquatique* et comprennent, comme exigences, un système de détection précoce (tel que décrit à l'article 1.4.7.) ainsi que des mesures de prévention de l'introduction de l'agent pathogène.

rapporté que le RSIV était responsable de pertes de production, de morbidités et de mortalités chez de nombreuses autres espèces (par exemple Chao *et al.*, 2004 ; Chen *et al.*, 2003 ; Girisha *et al.*, 2020 ; Ni *et al.*, 2021 ; Sumithra *et al.*, 2022).

La présence de l'ISKNV a été associée à de nombreux cas de maladies observées chez des poissons d'ornement (voir la revue de Johan & Zainathan, 2020). L'ISKNV a également été associé à des mortalités massives chez des espèces de poissons d'importance élevés pour la consommation humaine ; par exemple, la perche barramundi (Dong *et al.,* 2017 ; Kerddee *et al*., 2021), le tilapia (par exemple, Figueiredo *et al*., 2021 ; Ramírez-Paredes *et al*., 2021) et les mérous (par exemple, Chao *et al.*, 2004 ; Huang *et al.*, 2020).

Le TRBIV est responsable de maladies et de mortalités massives chez le turbot d'aquaculture en Chine (par exemple, Shi *et al*., 2010). Des mortalités pouvant atteindre 90 % ont été observées chez la perche barramundi à Taiwan (Tsai *et al.,* 2020).

Conclusion

Le critère est satisfait.

Critère n°4c. On a montré la présence de la maladie ou on dispose d'éléments de preuve scientifiques indiquant que la maladie affecterait la santé des animaux aquatiques sauvages avec de lourdes conséquences telles que, par exemple, une morbidité ou une mortalité à l'échelle de la population, une baisse de productivité ou des répercussions sur l'écologie.

Évaluation

Il y a un nombre limité d'informations sur la présence du RSIV, de l'ISKNV ou du TRBIV dans les populations de poissons sauvages et sur les conséquences en termes de morbidité, mortalité ou d'impact écologique. Il a été rapporté que l'ISKNV était à l'origine d'un épisode de mortalités massives chez des populations de cichlidés sauvages en Inde (Swaminathan *et al*., 2022) et qu'il a été également détecté chez diverses espèces de poissons sauvages apparemment en bonne santé (Wang *et al*., 2007).

Conclusion

Le critère n'est pas satisfait.

Références

ARMSTRONG, R. & FERGUSON, H. (1989). Systemic viral disease of the chromide cichlid Etroplus maculatus. *Diseases of Aquatic Organanisms,* **7**, 155-157.

ANDERSON, I.G., PRIOR, H.C., RODWELL, B.J. & HARRIS, G.O. (1993). Iridovirus-like virions in imported dwarf gourami (*Colisa lalia*) with systemic amoebiasis. *Australian Veterinary Journal,* **70(2)**, 66-67.

CHAO, C.B., CHEN, C.Y., LAI, Y.Y., LIN, C.S. & HUANG, H.T. (2004). Histological, ultrastructural, and in situ hybridization study on enlarged cells in the grouper *Epinephelus* hybrids infected with grouper iridovirus in Taiwan (TGIV). *Diseases of Aquatic Organisms*, **58**, 127–142.

CHEN, X.H., LIN, K.B. & WANG, X.W. (2003). Outbreaks of an iridovirus disease in maricultured large yellow croaker, *Larimichthys crocea* (Richardson), in China. *Journal of Fish Diseases*, **26**, 615-619.

CHINCHAR, V.R., HICK, P., INCE, I.A., JANCOVICH, J.K., MARSCHANG, R., QIN, Q., SUBRAMANIAM, K., WALTZEK, T.B., WHITTINGTON, R., WILLIAMS, T. & ZHANG, Q. (2017). ICTV Report Consortium ICTV Virus Taxonomy Profile: *Iridoviridae*. *Journal of Geneneral Virology,* **98**, 890–891.

CHINCHAR, V.G., HICK, P.H., HUANG, J., INCE, I.A., JANCOVICH, J.K., MARSCHANG, R., QIN, Q., SUBRAMANIAM, K., WALTZEK, T.B., WHITTINGTON, R., WILLIAMS, T. & ZHANG, Q. (2020) ICTV Virus Taxonomy Profile: *Iridoviridae*, *Journal of General Virology*, **98**, 890-891.

DO, J.W., CHA, S.J., KIM, J.S., AN, E.J., LEE, N.S., CHOI, H.J., LEE, C.H., PARK, M.S., KIM, J.W., KIM, Y.C. & PARK, J.W. (2005). Phylogenetic analysis of the major capsid protein gene of iridovirus isolates from cultured flounders *Paralichthys olivaceus* in Korea. *Diseases of Aquatic Organisms,* **64**, 193–200.

DONG, H.T., JITRAKORN, S., KAYANSAMRUAJ, P., PIRARATE, N., RODKHUM, C., RATTANAROJPONG, T., SENAPIN, S., SAKSMERPROME, V. (2017). Infectious spleen and kidney necrosis disease (ISKND) outbreaks in farmed barramundi (*Lates calcarifer*) in Vietnam. *Fish & Shellfish Immunology*, **68**, 65-73.

FIGUEIREDO, H.C.P., TAVARES, G.C., DORELLA, F.A., ROSA, J.C.C., MARCELINO, S.A.C., PIEREZAN, F. & PEREIRA, F.L. (2022). First report of infectious spleen and kidney necrosis virus in Nile tilapia in Brazil. *Transboundary and Emerging Diseases*, 69(5), 3008-3015.

GIRISHA, S.K., PUNEETH, T.G., NITHIN, M.S., NAVEEN KUMAR, B.T., AJAY, S.K., VINAY, T.N .& RAMESH, K.S. (2020). Red sea bream iridovirus disease (RSIVD) outbreak in Asian seabass (*Lates calcarifer*) cultured in open estuarine cages along the west coast of India: first report. *Aquaculture*, **520**, 734712. https://doi.org/10.1016/j.aquaculture.2019.734712

GO, J., WALTZEK, T.B., SUBRAMANIAM, K., YUN, S.C., GROFF, J.M., ANDERSON, I.G., CHONG, R., SHIRLEY, I., SCHUH, J.C.L., HANDLINGER, J.H., TWEEDIE, A. & WHITTINGTON, R.J. (2016). Complete genome analysis of the mandarin fish infectious spleen and kidney necrosis iridovirus. *Virology*, **291**, 126–139.

GO, J. & WHITTINGTON, R. (2006). Experimental transmission and virulence of a megalocytivirus (Family Iridoviridae) of dwarf gourami (*Colisa lalia*) from Asia in Murray cod (*Maccullochella peeli peeli*) in Australia. *Aquaculture*, **258**, 140-149.

GO, J. & WHITTINGTON, R.J. (2019). Experimental transmission of Infectious Spleen and Kidney Necrosis Virus (ISKNV) from freshwater ornamental fish to silver sweep *Scorpis lineolata*, an Australian marine fish. *Diseases of Aquatic Organisms*, **137(1)**, 1-21.

HE, J.G., DENG, M., WENG, S.P., LI, Z., ZHOU, S.Y., LONG, Q.X., WANG, X.Z. & CHAN, S.M. (2001). Complete genome analysis of the mandarin fish infectious spleen and kidney necrosis iridovirus. *Virology*, **291**, 126–139, doi: 10.1006/viro.2001.1208.

HE, J.G., ZENG, K., WENG, S.P. & CHAN, S.M. (2000). Systemic disease caused by an iridovirus-like agent in cultured mandarin fish *Siniperca chuatsi* (Basillewsky), in China, *Journal of Fish Diseases*, **23**, 219–222.

HE, J.G., ZENG, K., WENG, S.P. & CHAN, S.M. (2002), Experimental transmission, pathogenicity and physical-chemical properties of infectious spleen and kidney necrosis virus (ISKNV), *Aquaculture*, **204**, 11–24. doi: 10.1016/S0044-8486(01)00639-1.

HUANG, S.M., TU, C., TSENG, C.H., HUANG, C.C., CHOU, C.C., KUO, H.C. & CHANG, S.K. (2011). Genetic analysis of fish iridoviruses isolated in Taiwan during 2001-2009. *Archives of Virol*ogy, **156**, 1505-1515.

HUANG, Y., CAI, S., JIAN, J., LUI, G., & XU, L. (2020). Co-infection of infectious spleen and kidney necrosis virus and *Francisella* sp. in farmed pearl gentian grouper (♀*Epinephelus fuscoguttatus* ×♂*E. lanceolatus*) in China — A case report. *Aquaculture*, **526**, 735409.

INOUYE, K., YAMANO, K., MAENO, Y., NAKAJIMA, K., MATSUOKA, M., WADA, Y. & SORIMACHI, M. (1992). Iridovirus infection of cultured red sea bream, *Pagrus major*, *Fish Pathology*, **27**, 19–27.

JEONG, J.B., KIM, H.Y., JUN, L.J., LYU, J.H., PARK, N.G., KIM, J.K.& JEONG, H.D. (2008a). Outbreaks and risks of infectious spleen and kidney necrosis virus diseases in freshwater ornamental fishes, *Diseases of Aquatic Organisms*, **78**, 209–215. doi: 10.3354/dao01879.

JEONG, J., CHO, H., JUN, L., HONG, S., CHUNG, J. & JEONG, H. (2008b). Transmission of Iridovirus from freshwater ornamental fish (pearl gourami) to marine (rock bream). *Diseases of Aquatic Organisms*, **82(1)**, 27-36.

JOHAN, C.A.C.& ZAINATHAN, S.C. (2020). Megalocytiviruses in ornamental fish: A review. *Veterinary World*, **13**, 2565–2577.

JUNG, S.J., OH,M.J.. (2000). Iridovirus-like infection associated with high mortalities of striped beakperch, *Oplegnathus fasciatus* (Temminck et Schlegel), in southern coastal areas of the Korean peninsula, *Journal of Fish Diseases*, **23**, 223–226. doi: 10.1046/j.1365-2761.2000.00212.x.

KERDDEE, P., DINH-HUNG, N., THANH DONG, H., HIRONO, I., SOONTARA, C., AREECHON, N., SRISAPOOME, P. & KAYANSAMRUAJ, P. (2021). Molecular evidence for homologous strains of infectious spleen and kidney necrosis virus (ISKNV) genotype I infecting inland freshwater cultured Asian sea bass (*Lates calcarifer*) in Thailand. *Archives of Virology,* **166**, 3061–3074

KIM, K.H., CHOI, K.M., KANG, G., WOO, W.S., SOHN, M.Y., SON, H.J., YUN, D., KIM, D.H.& PARK, C.I. (2022). Development and Validation of a Quantitative Polymerase Chain Reaction Assay for the Detection of Red Sea Bream Iridovirus. *Fishes*, **7**, 236. https://doi.org/ 10.3390/fishes7050236

KODA, S.A., SUBRAMANIAM, K., POUDER D.B., YANONG, R.P. & WALTZEK, T.B. (2018). Phylogenomic characterization of red seabream iridovirus from Florida pompano *Trachinotus carolinus* maricultured in the Caribbean Sea. *Archives of Virology*, **164**, 1209-1212.

KURITA, J., NAKAJIMA, K., (2012), Megalocytiviruses, *Viruses*, **4(4)**, 521-538.

KAWATO, Y., CUMMINS, D.M., VALDETER, S., MOHR, P., ITO, T., MIZUNO, K., KAWAKAMI, H., WILLIAMS, L.M., CRANE, M.ST.J. & MOODY, N.J.G. (2021). Development of New Real-time PCR Assays for Detecting *Megalocytivirus* Across Multiple Genotypes. *Fish Pathology*, **56 (4)**, 177-186. doi.org/10.3147/jsfp.56.177

LAJIMIN, S., RAZAK, A.A., DENIL, D. J., RANSANGAN, J., ABDUL WAHID, M.E. & SADE, A. (2015). First detection of Megalocytivirus (*Iridoviridae*) in trash fish used for aquaculture feed in Sabah, Malaysia. *Int. J. of Aquatic Science*, 6(1): 54-66.

NAKAJIMA, K., MAENO, Y., HONDA, A., YOKOYAMA, K., TOORIYAMA, T. & MANABE, S. (1999). Effectiveness of a vaccine against red sea bream iridoviral disease in a field trial test, *Diseases of Aquatic Organisms*, **36(1)**, 73-5.

NI, S.Z., WANG, Y.J., HU, J. B., SHI, J., XU, Y., ZHOU, S.M., LI, J.J., HONG, B.H. & QIAN, D. (2021). Identification, histopathology, and phylogenetic analysis of an iridovirus from cultivated silver pomfret in Zhejiang Province, East China. *Aquaculture,* **530**,735619.

RAMÍREZ-PAREDES, J.G., PALEY, R.K., HUNT, W., FEIST, S.W., STONE, D.M., FIELD, T.R., HAYDON, D.J., ZIDDAH, P.A., NKANSA, M., GUILDER, J., GRAY, J., DUODU, S., PECKU, E.K., AWUNI,. J.A., WALLIS, T.S. & VERNER-JEFFREYS, D.W. (2021). First detection of infectious spleen and kidney necrosis virus (ISKNV) associated with massive mortalities in farmed tilapia in Africa. *Transboundry Emerging Diseases,* **68**, 1550–1563. https://doi.org/10.1111/tbed.13825

SHI, C.Y., WANG, Y.G., YANG, S.L., HUANG, J. & WANG, Q.Y. (2004). The first report of an iridovirus-like agent infection in farmed turbot, *Scophthalmus maximus*, in China. *Aquaculture*, **236**, 11-15. https://doi.org/10.1016/j.aquaculture.2003.11.007

SONG, J-Y., KITAMURA, S-I., JUNG, S-J., MIYADAI, T., TANAKA, S., FUKUDA, Y., KIM, S-R. & OH, M-J. (2008). Genetic variation and geographic distribution of megalocytiviruses. *Journal of Microbiology*, **46**, 29-33.

SUBRAMANIAM, K., SHARIFF, M., OMAR, A.R., HAIR-BEJO, M. & ONG, B.L. (2014). Detection and molecular characterisation of infectious spleen and kidney necrosis virus from major ornamental fish breeding states in peninsular Malaysia, *Journal of Fish Diseases*, **37**, 609–618, https://doi.org/10.1111/jfd.12152

SUBRAMANIAM, K., GOTESMAN, M., SMITH, C.E., STECKLER, N.K., KELLEY, K.L,, GROFF, J.M. & WALTZEK, T.B. (2016). *Megalocytivirus* infection in cultured Nile tilapia *Oreochromis niloticus*. *Diseases of Aquatic Organisms,* **119**, 253-258. https://doi.org/10.3354/dao02985

SUMITHRA, T.G., KRUPESHA SHARMA, S.R., NEELIMA, L., DHANUTHA, N.R., JOSHY, A., ANUSREE, V.N., GAYATHRI, S., RAGHU, R.K., PRAVEEN, N.D., THOMAS, S. & RAJESH, K.M. (2022). Red sea bream iridovirus infection in cage farmed Asian sea bass (*Lates calcarifer*): Insights into the pathology, epizootiology, and genetic diversity. *Aquaculture*, **548**, 737571. https://doi.org/10.1016/j.aquaculture.2021.737571

SWAMINATHAN, T.R., JOHNY, T.K., NITHIANANTHAM, S.R., SUDHAGAR, A., PRADHAN, P.K., SULUMANE RAMACHANDRA, K.S., NAIR, R.R., & SOOD, N. (2022). A natural outbreak of infectious spleen and kidney necrosis virus (ISKNV) threatens wild pearlspot, *Etroplus suratensis* in Peechi Dam in the Western Ghats biodiversity hotspot, India. *Transboundary and Emerging Diseases*, **69(5)**, 1595-1605. https://doi.org/10.1111/tbed.14494

TSAI, J.M., HUANG, S.L. & YANG, C.D. (2020). PCR Detection and Phylogenetic Analysis of *Megalocytivirus* Isolates in Farmed Giant Sea Perch *Lates calcarifer* in Southern Taiwan. Viruses, **12(6)**, 681. https://doi.org/10.3390/v12060681

WANG, Y.Q., LÜ, L., WENG, S.P., HUANG, J.N., CHAN, S.M.& HE, J.G. (2007). Molecular epidemiology and phylogenetic analysis of a marine fish infectious spleen and kidney necrosis viruslike (ISKNV-like) virus. A*rchives of Virology*, **152**, 763–773.

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Annexe 6. Point 5.3. – Article 9.3.1. du chapitre 9.3. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) »

CHAPITRE 9.3.

INFECTION À *HEPATOBACTER PENAEI* (HEPATOPANCRÉATITE NÉCROTISANTE)

Article 9.3.1.

Aux fins du *Code aquatique*, l'expression « infection à *Hepatobacter penaei* » (hépatopancréatite nécrosante) désigne une *infection* causée par *Candidatus* ~~*Hepatobacter penaei*~~ Hepatobacter penaei ; appartenant à la famille des Holosporaceae et à l'ordre des Rickettsiales ~~des alpha-protéobactéries~~ ; cet *agent pathogène* est une bactérie intracellulaire obligatoire. ~~La *maladie* est communément dénommée « hépatopancréatite nécrosante ».~~

Le *Manuel aquatique* contient des informations sur les méthodes de *diagnostic*.

[…]

Annexe 7. Point 5.4. – Articles 9.4.1. et 9.4.2. du chapitre 9.4. « Infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse »

CHAPITRE 9.4.

INFECTION PAR LE VIRUS DE LA NÉCROSE HYPODERMIQUE ET HÉMATOPOÏÉTIQUE INFECTIEUSE

Article 9.4.1.

Aux fins du *Code aquatique*, l'expression « infection par le virus de la nécrose hypodermique et hématopoïétique infectieuse » désigne une *infection* causée par le ~~penstyldensovirus~~ *penstylhamaparvovirus* 1 des décapodes, ~~couramment désigné comme le virus de la nécrose hypodermique et hématopoïétique~~ ; il s'agit d'un *agent pathogène* appartenant au genre *Penstyldensovirus* et à la famille des *Parvoviridae*.

Le *Manuel aquatique* contient des informations sur les méthodes de *diagnostic*.

Article 9.4.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. : la crevette bleue (*Penaeus stylirostris*),), la crevette géante tigrée (*Penaeus monodon*), la crevette ligubam du Nord (*Penaeus setiferus*), la crevette ~~à~~ pattes jaunes (*Penaeus californiensis*), ~~la crevette géante tigrée (*Penaeus monodon*), la crevette ligubam du Nord (*Penaeus setiferus*), la crevette bleue (*Penaeus stylirostris*)~~ et la crevette ~~à~~ pattes blanches (*Penaeus vannamei*).

[…]

Annexe 8. Point 5.5. – Article 9.5.2 du chapitre 9.5. « Infection par le virus de la myonécrose infectieuse »

CHAPITRE 9.5.

INFECTION PAR LE VIRUS DE LA MYONÉCROSE INFECTIEUSE

[…]

Article 9.5.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. : ~~la crevette tigrée brune (*Penaeus esculentus*)~~, la crevette ~~banana~~ banane (*Penaeus merguiensis*), la crevette tigrée sombre (*Penaeus esculentus*) et la crevette ~~à~~ pattes blanches (*Penaeus vannamei*).

[…]

Annexe 9. Point 5.5. – Article 10.9.2. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe »

CHAPITRE 10.9.

INFECTION PAR LE VIRUS DE LA VIRÉMIE PRINTANIÈRE DE LA CARPE

[…]

Article 10.9.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. :

Famille	Nom scientifique	Nom vernaculaire
Cyprinidae	*Abramis brama*	Brème (= brème d'eau douce)
	Aristichthys nobilis	Carpe à grosse tête
	Carassius auratus	Cyprin doré (= poisson rouge; = carpe dorée)
	Ctenopharyngodon idella	Carpe herbivore (= carpe chinoise; = carpe de roseau)
	Cyprinus carpio	Carpe commune (toutes les variétés et sous-espèces)
	Danio rerio	Poisson zèbre
	Notemigonus crysoleucas	[Golden shiner]
	Pimephales promelas	Tête de boule ~~Vairon à grosse tête~~ (=méné à grosse tête du Nord ; = vairon à grosse tête)
	Percocypris pingi	[Jinsha bass carp]
	Rutilus kutum	[Caspian white fish]
	Rutilus rutilus	Gardon
Siluridae	Silurus glanis	Silure glane

[…]

Annexe 10. Point 5.5. – Article 10.9.2. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe »

ÉVALUATION DE LA SENSIBILITÉ DE L'ESPÈCE DE POISSON À L'INFECTION PAR LE VIRUS DE LA VIRÉMIE PRINTANIÈRE DE LA CARPE

Contexte

En réponse à une requête de procéder à des évaluations de la sensibilité de *Percocypris pingi* à l'infection par le virus de la virémie printanière de la carpe, la Commission des animaux aquatiques a chargé le groupe *ad hoc* sur la sensibilité des espèces de poissons à une infection par une maladie listée par l'OIE (désigné ci-après comme le « groupe *ad hoc* ») d'examiner l'ensemble des éléments de preuve scientifiques et d'émettre des recommandations permettant à la Commission de prendre une décision.

Méthodologie

- Aux fins de la détermination de la sensibilité de *Percocypris pingi* à l'infection par le virus de la virémie printanière de la carpe, le groupe *ad hoc* a appliqué les critères d'évaluation tels que définis à l'article 1.5.3. du *Code aquatique*. Il a eu recours aux mêmes méthodologie et considérations que celles exposées dans le rapport d'évaluation de groupe *ad hoc* suivant : https://www.woah.org/app/uploads/2021/10/f-ahg-susceptibility-of-fish-november-2017.pdf

Évaluation de la sensibilité de l'hôte à l'infection par le virus de la virémie printanière de la carpe

Résultats

Le groupe *ad hoc* a estimé que *Percocypris pingi* satisfaisait aux critères d'inclusion dans la liste des espèces sensibles à l'infection par le virus de la virémie printanière de la carpe, conformément au chapitre 1.5. du *Code aquatique*. Il a proposé de l'ajouter dans l'article 10.9.2.

Tableau 1: Évaluation de la sensibilité de *Percocypris pingi* à l'infection par le virus de la virémie printanière de la carpe

Famille	**Nom scientifique**	**Nom vernaculaire**	**Étape 1 : Voie de transmission**	**Étape 2 : Identification de l'agent pathogène**	**Étape 3 : Preuve de l'infection**				**Résultat individuel**	**Références**
					A	**B**	**C**	**D**		
Catégorie 1										
Cyprinidae	*Percocypris pingi*	[Jinsha bass carp]	N	Culture suivie d'un séquençage	O	O	O	O	1	ZHENG *et al.*, 2018
Cyprinidae	*Percocypris pingi*	[Jinsha bass carp]	E/I						ND	ZHENG *et al.*, 2018

Acronymes figurant dans le tableau des évaluations
N : Apparition naturelle de l'infection.
E : Induction de l'infection au moyen de procédures expérimentales non invasives.
EI : Induction de l'infection au moyen de procédures expérimentales invasives.
OUI : La satisfaction au critère a été démontrée.
NON : La satisfaction au critère n'a pas été démontrée.
ND : La satisfaction au critère n'a pas été déterminée.

Commentaires du groupe *ad hoc*

- Bien qu'il n'y ait qu'une publication disponible aux fins de l'évaluation, le groupe *ad hoc* a estimé que les éléments de preuve apportés by Zheng *et al*. (2018) permettaient de classer la seule mais solide étude (présentant les caractéristiques pathologiques d'un foyer de la maladie apparu de façon naturelle, l'isolement du virus puis son identification par analyse séquentielle) dans la catégorie de résultat « 1 » et étaient suffisants pour conclure à la sensibilité de cette espèce, en l'absence d'éléments de preuve contradictoires.

- Le groupe *ad hoc* a également pris en compte que *Percocypris pingi* appartenait à la famille des Cyprinidae, dont plusieurs autres membres sont sensibles à cette infection. Les analyses séquentielles indiquent que le virus appartient au génogroupe Ia qui réunit les autres isolats chinois du virus de la virémie printanière de la carpe.

- Zheng *et al.* (2018) ont étudié un foyer de la maladie apparu naturellement. L'induction de l'infection au moyen de procédures *expérimentales* ne représentait qu'une partie de l'étude et n'a pas été prise en compte dans l'évaluation en raison de son caractère invasif. L'évaluation de la procédure expérimentale invasive n'a pas pu être poursuivie au-delà de l'étape 1 de l'article 1.5.4.

Références :

ZHENG, L.P., GENG, Y., YU, Z.H., WANG, K.Y., OU, Y.P., CHEN, D.F., HUANG, X.L., DENG, L.J., GAN, W.X., FANG, J., ZHONG, Z.J., LAI, W.M. (2018). First report of spring viremia of carp virus in *Percocypris pingi* in China. *Aquaculture.* **493**, 214-218.

CHAPITRE 10.X.

INFECTION PAR LE VIRUS DU TILAPIA LACUSTRE

Article 10.X.1.

Aux fins du *Code aquatique*, l'expression « infection par le virus du tilapia lacustre » désigne une *infection* causée par *Tilapia tilapinevirus*. Il s'agit d'un *agent pathogène* appartenant au genre *Tilapinevirus* et à la famille des *Amnoonviridae*.

Le *Manuel aquatique* contient des informations sur les méthodes de *diagnostic*.

Article 10.X.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles, conformément au chapitre 1.5. : [*Oreochromis aureus*, *Oreochromis niloticus* x *Oreochromis mossambicus*, *Sarotherodon galilaeus*, Tilapia du Mozambique (*Oreochromis mossambicus*), *Oreochromis niloticus*, *Tilapia zilli*, *Barbonymus schwanenfeldii*, *Tristramella simonis* et *Oreochromis niloticus* X *Oreochromis aureus*] (à l'étude).

Article 10.X.3.

Mesures pour l'importation, ou le transit par le territoire, de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus du tilapia lacustre

Les *produits issus d'animaux aquatiques* suivants ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus du tilapia lacustre, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus du tilapia lacustre :

1) [*produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive le virus du tilapia lacustre ;

2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive le virus du tilapia lacustre ;] (à l'étude)

3) huile de poisson ;

4) cuir élaboré à partir de peau de poisson.

Article 10.X.4.

Exigences pour l'auto-déclaration d'absence d'infection par le virus du tilapia lacustre

Un État membre peut déposer une auto-déclaration d'absence d'infection par le virus du tilapia lacustre pour l'intégralité du pays, une *zone* ou un *compartiment* conformément aux dispositions des articles 10.X.5. à 10.X.8., le cas échéant. L'auto-déclaration d'absence de maladie doit également être déposée conformément aux autres exigences pertinentes du *Code aquatique*, qui prévoient entre autres que l'État membre satisfasse aux conditions suivantes :

1) il respecte les dispositions du chapitre 3.1., et

2) il utilise des méthodes de *diagnostic* appropriées, telles que recommandées dans le *Manuel aquatique*, et

3) il répond à toutes les exigences mentionnées dans le chapitre 1.4. qui sont pertinentes pour l'auto-déclaration d'absence de maladie.

Article 10.X.5.

Pays indemne d'infection par le virus du tilapia lacustre

En cas de partage des étendues d'eau avec d'autres pays, un pays ne peut déposer une auto-déclaration d'absence d'infection par le virus du tilapia lacustre que si toutes les étendues d'eau partagées sont situées dans des pays ou des *zones* déclarés indemnes de cette *infection* (voir l'article 10.X.6.).

Comme indiqué à l'article 1.4.4., un État membre peut déposer une auto-déclaration d'absence d'infection par le virus du tilapia lacustre pour l'ensemble de son *territoire* s'il peut démontrer :

1) qu'aucune des *espèces sensibles* visées à l'article 10.X.2. n'est présente dans le pays et que les *conditions élémentaires de sécurité biologique* sont réunies sans discontinuer depuis au moins [six mois] ;

OU

2) qu'aucune infection par le virus du tilapia lacustre n'est apparue depuis au moins [10] ans, et :

   a) que l'État membre peut démontrer que les conditions propices à l'expression clinique de l'infection par le virus du tilapia lacustre sont réunies, comme indiqué au chapitre correspondant du *Manuel aquatique*, et

   b) que les *conditions élémentaires de sécurité biologique* telles que décrites dans le chapitre 1.4. sont réunies sans discontinuer depuis au moins [10] ans ;

OU

3) qu'une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre depuis au moins [deux] ans sans que la présence du virus du tilapia lacustre ait été décelée, et que les *conditions* élémentaires *de sécurité biologique* ont été réunies sans discontinuer au moins [un] an avant le commencement de la *surveillance ciblée* ;

OU

4) que le pays, après avoir déposé une auto-déclaration d'absence d'infection par le virus du tilapia lacustre, a perdu son statut indemne par suite de la détection du virus du tilapia lacustre, mais que les conditions suivantes sont remplies :

   a) dès la détection du virus du tilapia lacustre, le secteur touché a été déclaré *zone infectée* et une *zone de protection* a été établie, et

   b) les populations touchées par l'*infection* de la *zone infectée* ont été abattues et éliminées par des moyens réduisant autant que possible la probabilité de nouvelle transmission du virus du tilapia lacustre, et les opérations appropriées de *désinfection* (décrites au chapitre 4.4.) ont été menées à bien et suivies d'une période de *vide sanitaire* comme indiqué au chapitre 4.7., et

   c) les *conditions élémentaires de sécurité biologique* existant antérieurement ont été réexaminées, et sont en place sans discontinuer, avec les modifications éventuellement nécessaires, depuis l'éradication de l'infection par le virus du tilapia lacustre, et

   d) une *surveillance ciblée*, comme décrit au chapitre 1.4., est exercée :

      i) depuis au moins [deux] ans sur les *espèces sensibles* d'élevage et sauvages sans que la présence du virus du tilapia lacustre ait été décelée, ou

ii) depuis au moins [un] an sans que la présence du virus du tilapia lacustre ait été décelée dans le cas où les *établissements d'aquaculture* touchés ne présentent aucun lien épidémiologique avec des populations sauvages d'*espèces sensibles*.

Entre-temps, tout ou partie du pays, à l'exclusion des *zones infectées* et des *zones de protection*, peut être déclaré *zone* indemne sous réserve que les conditions énoncées au point 2 de l'article 10.X.6. soient remplies.

Article 10.X.6.

Zone indemne d'infection par le virus du tilapia lacustre

En cas d'extension au-delà du *territoire* de plus d'un pays, une *zone* ne peut être déclarée indemne d'infection par le virus du tilapia lacustre que si l'ensemble des *Autorités compétentes* concernées confirment que toutes les conditions voulues sont remplies.

Comme indiqué dans l'article 1.4.4., un État membre peut déposer une auto-déclaration d'absence d'infection par le virus du tilapia lacustre pour une *zone* établie sur son *territoire* s'il peut démontrer :

1) qu'aucune des *espèces sensibles* visées à l'article 10.X.2. n'est présente et que les *conditions* élémentaires *de sécurité biologique* sont réunies sans discontinuer depuis au moins [six mois] ;

OU

2) qu'aucune infection par le virus du tilapia lacustre n'est apparue depuis au moins [dix] ans, et :

   a) que l'État membre peut démontrer que les conditions propices à l'expression clinique de l'infection par le virus du tilapia lacustre sont réunies, comme décrit à l'article 1.4.8. du chapitre 1.4., et

   b) que les *conditions élémentaires de sécurité biologique* telles que décrites dans le chapitre 1.4. sont réunies sans discontinuer dans la *zone* depuis au moins [dix] ans ;

OU

3) qu'une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre dans la *zone* depuis au moins [deux] ans sans que la présence du virus du tilapia lacustre ait été décelée, et que les *conditions élémentaires de sécurité biologique* ont été réunies sans discontinuer au moins [un] an avant le commencement de la *surveillance ciblée* ;

OU

4) que le pays, après avoir déposé une auto-déclaration d'absence d'infection par le virus du tilapia lacustre pour une *zone*, a perdu son statut indemne par suite de la détection du virus du tilapia lacustre dans cette *zone*, mais que les conditions suivantes sont remplies :

   a) dès la détection du virus du tilapia lacustre, le secteur touché a été déclaré *zone infectée* et une *zone de protection* a été établie, et

   b) les populations touchées par l'*infection* de la *zone infectée* ont été abattues et éliminées par des moyens réduisant autant que possible la probabilité de nouvelle transmission du virus du tilapia lacustre, et les opérations appropriées de *désinfection* (décrites au chapitre 4.4.) ont été menées à bien et suivies d'une période de *vide sanitaire* comme indiqué au chapitre 4.7., et

   c) les *conditions élémentaires de sécurité biologique* existant antérieurement ont été réexaminées, et sont en place sans discontinuer, avec les modifications éventuellement nécessaires, depuis l'éradication de l'infection par le virus du tilapia lacustre, et

   d) une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre depuis au moins [deux] ans sans que la présence du virus du tilapia lacustre ait été décelée.

Article 10.X.7.

Compartiment indemne d'infection par le virus du tilapia lacustre

Comme indiqué dans l'article 1.4.4., un État membre peut déposer une auto-déclaration d'absence d'infection par le virus du tilapia lacustre pour un *compartiment* établi sur son *territoire* s'il peut démontrer :

1) qu'une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre dans le *compartiment* depuis au moins [un] an sans que la présence du virus du tilapia lacustre ait été décelée, et que les *conditions élémentaires de sécurité biologique* ont été réunies sans discontinuer au moins [un] an avant le commencement de la *surveillance ciblée* ;

OU

2) que le pays, après avoir déposé une auto-déclaration d'absence d'infection par le virus du tilapia lacustre pour un *compartiment*, a perdu son statut indemne par suite de la détection du virus du tilapia lacustre dans ce *compartiment*, mais que les conditions suivantes sont remplies :

   a) tous les *animaux aquatiques* détenus dans le *compartiment* ont été abattus et éliminés par des moyens réduisant autant que possible la probabilité de nouvelle transmission du virus du tilapia lacustre, les opérations de *désinfection* appropriées (décrites au chapitre 4.4.) ont été menées à bien, et un *vide sanitaire* a été mis en place dans le *compartiment* comme indiqué au chapitre 4.7., et

   b) les *conditions élémentaires de sécurité biologique* existant antérieurement, incluant le *plan de sécurité biologique* applicable au *compartiment*, ont été réexaminées, et sont en place sans discontinuer, avec les modifications éventuellement nécessaires, depuis le repeuplement avec des *animaux aquatiques* issus d'une source agréée indemne d'agents pathogènes, dans le respect des exigences mentionnées dans l'article 10.X.9. ou dans l'article 10.X.10. selon le cas, et

   c) une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre dans le *compartiment* depuis au moins [un] an sans que la présence du virus du tilapia lacustre ait été décelée.

Article 10.X.8.

Maintien du statut indemne

Un pays, une *zone* ou un *compartiment* qui est déclaré indemne d'infection par le virus du tilapia lacustre conformément aux dispositions prévues aux articles 10.X.4. à 10.X.7. (selon le cas) peut conserver son statut indemne au regard de cette *infection* sous réserve que les exigences mentionnées à l'article 1.4.15. soient constamment respectées.

Article 10.X.9.

Importation d'animaux aquatiques ou de produits issus d'animaux aquatiques à partir d'un pays, d'une zone ou d'un compartiment déclaré indemne d'infection par le virus du tilapia lacustre

Lors d'une importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 10.X.2., ou de *produits issus d'animaux aquatiques* dérivés de ces espèces, à partir d'un pays, d'une *zone* ou d'un *compartiment* déclaré indemne d'infection par le virus du tilapia lacustre, l'*Autorité compétente* du *pays importateur* doit exiger que l'envoi soit accompagné d'un *certificat sanitaire international applicable aux animaux aquatiques* délivré par l'*Autorité compétente* du *pays exportateur*. Le *certificat sanitaire international applicable aux animaux aquatiques* doit attester que le lieu de production des *animaux aquatiques* ou des *produits issus d'animaux aquatiques* est un pays, une *zone* ou un *compartiment* déclaré indemne d'infection par le virus du tilapia lacustre sur la base des procédures définies par les articles 10.X.5., 10.X.6. ou 10.X.7. (selon le cas) et 10.X.8.

Le *certificat sanitaire international applicable aux animaux aquatiques* doit être conforme au modèle reproduit au chapitre 5.11.

Le présent article ne s'applique pas aux *produits issus d'animaux aquatiques* visés à l'article 10.X.3.

Article 10.X.10.

Importation d'animaux aquatiques à des fins d'aquaculture, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par le virus du tilapia lacustre

Lors de l'importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 10.X.2. à des fins d'*aquaculture* à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par le virus du tilapia lacustre, l'*Autorité compétente* du *pays importateur* doit apprécier le *risque* associé à cette importation conformément au chapitre 2.1. et prendre en considération les mesures d'atténuation du *risque* prévues aux points 1 et 2 ci-dessous.

1) Si l'objectif est le grossissement et la récolte des *animaux aquatiques* importés, il convient d'appliquer les principes suivants :

   a) la livraison directe et le maintien à vie des *animaux aquatiques* importés dans une installation de *quarantaine*, et

   b) avant leur départ de *quarantaine* (qu'il s'agisse de l'installation d'origine ou d'une autre installation de *quarantaine* jusqu'à laquelle les animaux ont été transportés dans des conditions de *sécurité biologique* adéquates), la mise à mort et la transformation des *animaux aquatiques* en l'un ou plusieurs des *produits issus d'animaux aquatiques* visés à l'article 10.X.3. ou en l'un des autres produits autorisés par l'*Autorité compétente*, et

   c) le traitement de toute l'eau utilisée pour le transport ainsi que de tous les équipements, effluents et déchets afin d'inactiver le virus du tilapia lacustre conformément aux chapitres 4.4., 4.8. et 5.5.

OU

2) Si l'objectif est l'établissement d'une nouvelle population à des fins d'*aquaculture*, il convient d'appliquer les principes suivants :

   a) dans le *pays exportateur* :

      i) identifier les populations sources potentielles et évaluer les données sanitaires des *animaux aquatiques* qui les composent ;

      ii) tester les populations sources conformément au chapitre 1.4. et sélectionner une population de géniteurs (F-0) d'*animaux aquatiques* présentant un statut sanitaire élevé au regard de l'infection par le virus du tilapia lacustre ;

   b) dans le *pays importateur* :

      i) placer la population de géniteurs (F-0) importée dans une installation de *quarantaine* ;

      ii) tester la population F-0 aux fins de la recherche du virus du tilapia lacustre conformément au chapitre 1.4. afin de déterminer si elle constitue une population de géniteurs adéquate ;

      iii) produire une première génération (F-1) en *quarantaine* ;

      iv) élever la population F-1 dans une installation de *quarantaine* pendant une durée suffisante, et dans des conditions propices, pour permettre l'expression clinique de l'infection par le virus du tilapia lacustre, et prélever des échantillons et tester la présence du virus du tilapia lacustre chez cette population conformément au chapitre 1.4. du *Code aquatique* et au chapitre X.X.6. du *Manuel aquatique* ;

      v) si le virus du tilapia lacustre n'est pas détecté dans la population F-1, cette dernière pourra être reconnue indemne d'infection par le virus du tilapia lacustre et libérée de sa *quarantaine* ;

      vi) si le virus du tilapia lacustre est détecté dans la population F-1, cette dernière ne sera pas libérée de sa *quarantaine*, et sera tuée puis éliminée de manière biosécurisée, conformément au chapitre 4.8.

Article 10.X.11.

Importation d'animaux aquatiques ou de produits issus d'animaux aquatiques à des fins de transformation ultérieure en vue de la consommation humaine, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par le virus du tilapia lacustre

Lors de l'importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 10.X.2., ou de *produits issus d'animaux aquatiques* dérivés de ces espèces, à des fins de transformation ultérieure en vue de la consommation humaine à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par le virus du tilapia lacustre, l'*Autorité compétente* du *pays importateur* doit apprécier le *risque* associé à cette importation et, si la situation le justifie, exiger :

1) la livraison directe du chargement, ainsi que son maintien, dans des installations de *quarantaine* ou d'entreposage jusqu'à ce qu'il soit transformé en l'un des produits visés à l'article 10.X.3. ou au point 1 de l'article 10.X.14. ou en l'un des autres produits autorisés par l'*Autorité compétente*, et

2) le traitement de toute l'eau (y compris sous forme de glace) ainsi que de l'ensemble des équipements, *conteneurs* et matériaux d'emballage utilisés lors du transport dans des conditions permettant d'inactiver le virus du tilapia lacustre ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4., 4.8. et 5.5., et

3) le traitement de tous les effluents et de tous les déchets dans des conditions permettant d'inactiver le virus du tilapia lacustre ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4. et 4.8.

Lorsqu'ils l'estiment nécessaire, les États membres peuvent prendre des mesures au niveau national, visant à limiter les *risques* associés à l'utilisation des *animaux aquatiques* ou des *produits issus d'animaux aquatiques* susvisés à des fins autres que la consommation humaine.

Article 10.X.12.

Importation d'animaux aquatiques ou de produits issus d'animaux aquatiques destinés à des fins autres que la consommation humaine, parmi lesquelles l'alimentation animale, les usages agricoles, industriels ou pharmaceutiques et la recherche, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par le virus du tilapia lacustre

Lors de l'importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 10.X.2., ou de *produits issus d'animaux aquatiques* dérivés de ces espèces, destinés à des fins autres que la consommation humaine, parmi lesquelles l'alimentation animale, les usages agricoles, industriels ou pharmaceutiques et la recherche, à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par le virus du tilapia lacustre, l'*Autorité compétente* du *pays importateur* doit exiger :

1) la livraison directe du chargement, ainsi que son maintien, dans des installations de *quarantaine* ou d'entreposage jusqu'à ce qu'il soit transformé en l'un des produits visés à l'article 10.X.3. ou en l'un des produits autorisés par l'*Autorité compétente*, et

2) le traitement de toute l'eau (y compris sous forme de glace) ainsi que de l'ensemble des équipements, *conteneurs* et matériaux d'emballage utilisés lors du transport dans des conditions permettant d'inactiver le virus du tilapia lacustre ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4., 4.8. et 5.5., et

3) le traitement de tous les effluents et de tous les déchets dans des conditions permettant d'inactiver le virus du tilapia lacustre ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4. et 4.8.

Article 10.X.13.

Importation d'animaux aquatiques destinés à des laboratoires ou à des établissements zoologiques, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par le virus du tilapia lacustre

Lors d'une importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 10.X.2. qui sont destinés à des laboratoires ou à des établissements zoologiques à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par le virus du tilapia lacustre, l'*Autorité compétente* du *pays importateur* doit veiller :

1) à la livraison directe du chargement, ainsi qu'à son maintien, dans des installations de *quarantaine* agréées par l'*Autorité compétente*, et

2) au traitement de toute l'eau (y compris sous forme de glace) ainsi que de l'ensemble des équipements, *conteneurs* et matériaux d'emballage utilisés lors du transport dans des conditions permettant d'inactiver le virus du tilapia lacustre ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4., 4.8. et 5.5., et

3) au traitement de tous les effluents et déchets issus des installations de *quarantaine* des laboratoires ou des établissements zoologiques dans des conditions permettant d'inactiver le virus du tilapia lacustre ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4. et 4.8., et

4) à l'élimination des cadavres conformément au chapitre 4.8.

Article 10.X.14.

Importation (ou transit par le territoire) de produits issus d'animaux aquatiques pour la vente au détail de marchandises destinées à la consommation humaine, indépendamment du statut sanitaire du pays exportateur ou de la zone ou du compartiment d'exportation au regard de l'infection par le virus du tilapia lacustre

1) [Quel que soit le statut sanitaire du *pays exportateur* ou de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus du tilapia lacustre, les *Autorités compétentes* ne doivent imposer aucune condition liée au virus du tilapia lacustre quand elles autorisent l'importation (ou le transit par leur *territoire*) des *marchandises* suivantes qui ont été préparées et emballées pour la vente au détail lorsqu'elles satisfont aux dispositions prévues à l'article 5.4.2. :

   a) filets ou de darnes ou pavés de poisson (à l'état réfrigéré)] (à l'étude).

   Certaines hypothèses ont été posées concernant l'évaluation de la sécurité sanitaire des *produits issus d'animaux aquatiques* susvisés. Les États membres doivent donc se référer à ces hypothèses, figurant à l'article 5.4.2., et estimer si ces dernières s'appliquent à leur situation.

   Lorsqu'ils l'estiment nécessaire, les États membres peuvent prendre des mesures au niveau national, visant à limiter les *risques* associés à l'utilisation des *produits issus d'animaux aquatiques* susvisés à des fins autres que la consommation humaine.

2) Lors d'une importation de *produits issus d'animaux aquatiques* appartenant à l'une des espèces visées à l'article 10.X.2., à l'exclusion de ceux visés au point 1 qui précède, à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par le virus du tilapia lacustre, l'*Autorité compétente* du *pays importateur* doit apprécier le *risque* associé à cette importation et appliquer des mesures appropriées visant à atténuer ce *risque*.

———————

Annexe 12. Point 5.7. – Article 11.2.2. du chapitre 11.2. « Infection à *Bonamia exitiosa* »

CHAPITRE 11.2.

INFECTION À *BONAMIA EXITIOSA*

[…]

Article 11.2.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. : ~~l'huître argentine (~~*Ostrea puelchana*~~)~~, ~~l'huître plate australienne (~~*Ostrea angasi*~~)~~, l'huître plate chilienne (*Ostrea chilensis*), *Ostrea equestris*, l'huître creuse américaine (*Crassostrea virginica*), l'huître plate européenne (*Ostrea edulis*), l'huître plate indigène (*Ostrea lurida*) et ~~l'huître creuse de Suminoe (~~*Magallana* (syn. *Crassostrea*) *ariakensis*~~)~~.

[…]

Annexe 13. Point 5.7. – Article 11.3.2. du chapitre 11.3. « Infection à *Bonamia ostreae* »

CHAPITRE 11.3.

INFECTION À *BONAMIA OSTREAE*

[…]

Article 11.3.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. : l'huître plate européenne (*Ostrea edulis*), l'huître plate chilienne (*Ostrea chilensis*) et *Magallana* (syn. *Crassostrea*) *ariakensis*.

[…]

Annexe 14. Point 5.8. – Articles 11.4.1. et 11.4.2. du chapitre 11.4. « Infection à *Marteilia refringens* »

CHAPITRE 11.4.

INFECTION À *MARTEILIA REFRINGENS*

Article 11.4.1.

Aux fins du *Code aquatique*, l'expression « infection à *Marteilia refringens* » désigne une *infection* causée ~~exclusivement~~ par *Marteilia refringens* : il s'agit d'un *agent pathogène* appartenant à la famille des Marteiliidae.

Le *Manuel aquatique* contient des informations sur les méthodes de *diagnostic*.

Article 11.4.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent à la moule commune (*Mytilus edulis*), à l'huitre naine (*Ostrea stentina*), à l'huître plate européenne *(Ostrea edulis)*, au couteau d'Europe (*Solen marginatus*), à *Xenostrobus securis*, ~~à l'huître plate australienne (*Ostrea angasi*), à l'huître plate argentine (*Ostrea puelchana*), à l'huître plate chilienne (*Ostrea chilensis*), à la moule commune (*Mytilus edulis*)~~ et à la moule méditerranéenne (*Mytilus galloprovincialis*) et à la petite praire (*Chamelea gallina*). ~~Ces recommandations concernent également toutes les autres espèces sensibles visées dans le *Manuel aquatique* lorsqu'elles font l'objet d'échanges internationaux.~~

[…]

Annexe 15. Point 5.9. – Modèles d'articles 11.X.9. – 11.X.14.
destinés aux chapitres spécifiques aux maladies des mollusques

Modèles d'articles 11.X.9.à 11.X.14. destinés aux chapitres spécifiques aux maladies des mollusques

CHAPITRE 11.X.

INFECTION PAR [L'AGENT PATHOGÈNE X]

[...]

Article 11.X.9.

Importation d'animaux aquatiques ~~et~~ ou de produits issus d'animaux aquatiques à partir d'un pays, d'une zone ou d'un compartiment déclaré indemne d'infection par [l'agent pathogène X]

Lors d'une importation d'*animaux aquatiques* ~~et de *produits issus d'animaux aquatiques*~~ appartenant à l'une des espèces visées à l'article 11.X.2., ou de *produits issus d'animaux aquatiques* ~~appartenant à des espèces visées à l'article 11.2.2~~. dérivés de ces espèces, à partir d'un pays, d'une *zone* ou d'un *compartiment* déclaré indemne d'infection par [l'agent pathogène X], l'*Autorité compétente* du *pays importateur* doit exiger que l'envoi soit accompagné d'un *certificat sanitaire international applicable aux animaux aquatiques* délivré par l'*Autorité compétente* du *pays exportateur*.~~, ou par un *agent certificateur* agréé par le *pays importateur*~~, ~~et attestant~~ Le *certificat sanitaire international applicable aux animaux aquatiques* doit attester que le lieu de production des *animaux aquatiques* ~~et~~ ou des *produits issus d'animaux aquatiques* est un pays, une *zone* ou un *compartiment* déclaré indemne d'infection par [l'agent pathogène X] sur la base des procédures définies par les articles 11.X.4~~5~~., ~~ou~~ 11.X.~~5.~~6. ou 11.X.7. (selon le cas) et 11.X.~~6~~8.

Le *certificat sanitaire international applicable aux animaux aquatiques* doit être conforme au modèle reproduit au chapitre 5.11.

Le présent article ne s'applique pas aux ~~*marchandises*~~ *produits issus d'animaux aquatiques* visé~~e~~s au point 1 de l'article 11.X.3.

Article 11.X.10.

Importation d'animaux aquatiques à des fins d'aquaculture, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par [l'agent pathogène X]

Lors de l'importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 11.X.2. à des fins d'*aquaculture* à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par [l'agent pathogène X], l'*Autorité compétente* du *pays importateur* doit apprécier le *risque* associé à cette importation conformément au chapitre 2.1. et prendre en considération les mesures d'atténuation du *risque* prévues aux points 1 et 2 ci-dessous.

1) Si l'objectif est le grossissement et la récolte des *animaux aquatiques* importés, il convient d'appliquer les principes suivants :

   a) la livraison directe et le maintien à vie des *animaux aquatiques* importés dans une installation de *quarantaine*~~, et~~ ;

   b) avant leur départ de *quarantaine* (qu'il s'agisse de l'installation d'origine ou d'une autre installation de *quarantaine* jusqu'à laquelle les animaux ont été transportés dans des conditions de *sécurité biologique* adéquates), la mise à mort et la transformation des *animaux aquatiques* en l'un ou plusieurs des *produits issus d'animaux aquatiques* visés au point 1 de l'article 11.X.3. ou en l'un des autres produits autorisés par l'*Autorité*

compétente, et

c) le traitement de toute l'eau utilisée pour le transport ainsi que de tous les équipements, effluents et déchets afin d'inactiver [l'agent pathogène X] conformément aux chapitres 4.4., 4.8. et 5.5.

OU

2) Si l'objectif est l'établissement d'une nouvelle population à des fins d'*aquaculture*, il convient d'appliquer les principes suivants :

a) dans le *pays exportateur* :

i) identifier les populations sources potentielles et évaluer les données sanitaires des *animaux aquatiques* qui les composent ;

ii) tester les populations sources conformément au chapitre 1.4. et sélectionner une population de géniteurs (F-0) d'*animaux aquatiques* présentant un statut sanitaire élevé au regard de l'infection par [l'agent pathogène X] ;

b) dans le *pays importateur* :

i) placer la population de géniteurs (F-0) importée dans une installation de *quarantaine* ;

ii) tester la population F-0 aux fins de la recherche de [l'agent pathogène X] conformément au chapitre 1.4. afin de déterminer si elle constitue une population de géniteurs adéquate ;

iii) produire une première génération (F-1) en *quarantaine* ;

iv) élever la population F-1 dans une installation de *quarantaine* dans des conditions propices à l'expression des signes cliniques de l'infection par [l'agent pathogène X] (tels qu'ils sont décrits au chapitre 2.4.X. du *Manuel aquatique*), et tester la présence de [l'agent pathogène X] chez cette population conformément au chapitre 1.4. ;

v) si [l'agent pathogène X] n'est pas détecté dans la population F-1, cette dernière pourra être reconnue indemne d'infection par [l'agent pathogène X] et libérée de sa *quarantaine* ;

vi si [l'agent pathogène X] est détecté dans la population F-1, cette dernière ne sera pas libérée de sa *quarantaine,* et sera tuée puis éliminée de manière biosécurisée,

Article 11.X.11.

Importation d'animaux aquatiques ~~et~~ ou de produits issus d'animaux aquatiques à des fins de transformation ultérieure en vue de la consommation humaine, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par [l'agent pathogène X]

Lors de l'importation, à des fins de transformation ultérieure en vue de la consommation humaine, d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 11.X.2., ou de *produits issus d'animaux aquatiques* dérivés de ces espèces, à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par [l'agent pathogène X], l'*Autorité compétente* du *pays importateur* doit apprécier le *risque* associé à cette importation et, si la situation le justifie, exiger :

1) la livraison directe du chargement et son entreposage dans des installations de *quarantaine* ou de confinement jusqu'au moment de sa transformation en l'un des produits visés au point 1 de l'article 11.X.3. ou ~~produits décrits~~ au point 1 de l'article 11.X.12., ou encore en l'un des autres produits autorisés par l'*Autorité compétente*, et

2) le traitement de toute l'eau (y compris sous forme de glace) ainsi que de l'ensemble des équipements, *conteneurs* et matériaux d'emballage utilisés lors du transport et ~~celui de tous les effluents et déchets résultant des opérations de transformation~~ dans des conditions permettant d'inactiver [l'agent pathogène X] ou de les éliminer de manière ~~à empêcher leur contact avec des *espèces sensibles*~~ biosécurisée conformément aux chapitres 4.4., 4.8. et 5.5., et

3) le traitement de tous les effluents et de tous les déchets dans des conditions permettant d'inactiver [l'agent pathogène X] ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4. et 4.8.

Lorsqu'ils l'estiment nécessaire, les États membres peuvent prendre des mesures au niveau national, visant à limiter les *risques* associés à l'utilisation des ~~*marchandises*~~ *animaux aquatiques* ou des *produits issus d'animaux aquatiques* susvisés à des fins autres que la consommation humaine.

Article 11.X.12.

Importation d'animaux aquatiques ou de produits issus d'animaux aquatiques destinés à des fins autres que la consommation humaine, parmi lesquelles l'alimentation animale, les usages agricoles, industriels ou pharmaceutiques et la recherche, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par [l'agent pathogène X]

Lors de l'importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 11.X.2., ou de *produits issus d'animaux aquatiques* dérivés de ces espèces, destinés à des fins autres que la consommation humaine, parmi lesquelles l'alimentation animale, les usages agricoles, industriels ou pharmaceutiques et la recherche, à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par [l'agent pathogène X], l'*Autorité compétente* du *pays importateur* doit exiger :

1) la livraison directe du chargement, ainsi que son maintien, dans des installations de *quarantaine* en vue d'y être abattu et transformé en des produits autorisés par l'*Autorité* compétente, et

2) le traitement de toute l'eau (y compris sous forme de glace) ainsi que de l'ensemble des équipements, *conteneurs* et matériaux d'emballage utilisés lors du transport ~~résultant des opérations de transformation~~ dans des conditions permettant d'inactiver [l'agent pathogène X] ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4., 4.8. et 5.5., et

3) le traitement de tous les effluents et de tous les déchets dans des conditions permettant d'inactiver [l'agent pathogène X] ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4. et 4.8.

~~Le présent article ne s'applique pas aux *marchandises* visées au point 1 de l'article 11.2.3.~~

Article 11.X.13.

[**Note** : il s'agit d'un nouvel article devant être aligné sur les autres chapitres spécifiques aux maladies figurant dans le *Code aquatique*.]

Importation d'animaux aquatiques destinés à des laboratoires ou à des établissements zoologiques, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par [l'agent pathogène X]

Lors d'une importation d'*animaux aquatiques* appartenant à l'une des espèces visées à l'article 11.X.2. qui sont destinés à des laboratoires ou à des établissements zoologiques à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par [l'agent pathogène X], l'*Autorité compétente* du *pays importateur* doit veiller :

1) à la livraison directe du chargement, ainsi qu'à son maintien, dans des installations de *quarantaine* agréées par l'*Autorité compétente*, et

2) au traitement de toute l'eau (y compris sous forme de glace) ainsi que de l'ensemble des équipements, *conteneurs* et matériaux d'emballage utilisés lors du transport dans des conditions permettant d'inactiver [l'agent pathogène X] ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4., 4.8. et 5.5., et

3) au traitement de tous les effluents et déchets issus des installations de *quarantaine* des laboratoires ou des établissements zoologiques dans des conditions permettant d'inactiver [l'agent pathogène X] ou de les éliminer de manière biosécurisée conformément aux chapitres 4.4. et 4.8., et

4) à l'élimination des cadavres conformément au chapitre 4.8.

Article 11.X.~~13~~14.

Importation (ou transit par le territoire) ~~d'animaux aquatiques et~~ de produits issus d'animaux aquatiques pour la vente au détail de marchandises destinées à la consommation humaine, à partir d'un pays, d'une zone ou d'un compartiment non déclaré indemne d'infection par [l'agent pathogène X]

1) Quel que soit le statut sanitaire du *pays exportateur* ou de la *zone* ou du *compartiment* d'exportation au regard de l'infection par [l'agent pathogène X], les *Autorités compétentes* ne doivent imposer aucune condition liée à l'infection par [l'agent pathogène X] quand elles autorisent l'importation ou le transit par leur *territoire* des *marchandises* suivantes qui ont été préparées et emballées pour la vente au détail lorsqu'elles satisfont aux conditions énoncées à l'article 5.4.2. :

   a) [...].

   Certaines hypothèses ont été posées concernant l'évaluation de la sécurité sanitaire des *produits issus d'animaux aquatiques* susvisés. Les États membres doivent donc se référer à ces hypothèses, figurant à l'article 5.4.2., et estimer si ces dernières s'appliquent à leur situation.

   Lorsqu'ils l'estiment nécessaire, les États membres peuvent prendre des mesures au niveau national, visant à limiter les *risques* associés à l'utilisation ~~du type de *marchandise*~~ des *produits issus d'animaux aquatiques* susvisés à des fins autres que la consommation humaine.

2) Lors d'une importation ~~d'*animaux aquatiques* et~~ de *produits issus d'animaux aquatiques*, à l'exclusion de ceux visés au point 1 qui précède, dérivés d'une des espèces visées à l'article 11.X.2. à partir d'un pays, d'une *zone* ou d'un *compartiment* non déclaré indemne d'infection par l'[agent pathogène X], l'*Autorité compétente* du *pays importateur* doit apprécier le *risque* associé à cette importation et appliquer des mesures appropriées visant à réduire ce *risque*.

Annexe 16. Point 5.10. – Questionnaire à l'intention des Membres pour la révision du chapitre 4.3. « Application de la compartimentation »

QUESTIONNAIRE À L'INTENTION DES MEMBRES

RÉVISION DU CHAPITRE 4.3. « APPLICATION DE LA COMPARTIMENTATION »

Un questionnaire a été élaboré par la Commission des normes sanitaires pour les animaux aquatiques. Il est destiné aux Membres pour avis.

Contexte

La nécessité de réviser le chapitre 4.3. « Application de la compartimentation » a été prise en compte par la Commission des animaux aquatiques, il y a un certain temps, par son ajout dans la partie du plan de travail relative à la révision progressive du Titre 4 du *Code aquatique*. Cette révision a été identifiée comme étant nécessaire lors de la Conférence mondiale de l'OIE sur la santé des animaux aquatiques en 2019. Elle figure dans les activités de la Stratégie de l'OIE pour la santé des animaux aquatiques (2021-2025).

Lors de sa réunion en février 2022, la Commission des animaux aquatiques a identifié la révision du chapitre 4.3. « Application de la compartimentation » comme étant une priorité dans le cadre de la révision progressive du Titre 4 du *Code aquatique*. Le chapitre sera modifié afin qu'il traite uniquement de la compartimentation, que les orientations destinées aux Membres soient améliorées et qu'il soit en ligne avec les chapitres nouveaux ou révisés tels que le chapitre 4.1. « Sécurité biologique dans les établissements d'aquaculture ». Dans cette perspective sont apparues comme particulièrement pertinentes la version révisée et récemment adoptée du chapitre 1.4. sur la surveillance et les modèles d'articles X.X.4. à X.X.8 sur la déclaration du statut indemne destinés aux chapitres spécifiques aux maladies, qui incluent des références spécifiques aux exigences pour la démonstration et le maintien du statut indemne à l'échelle du compartiment. La révision du chapitre 4.3. « Application de la compartimentation » apparait comme une prochaine étape appropriée.

La version actuelle du chapitre 4.2. « Zonage et compartimentation » a été adoptée en 1995 et révisée pour la dernière fois en 2010 alors que le chapitre 4.3. « Application de la compartimentation » a été adoptée en 2010 et révisé pour la dernière fois en 2016. L'expérience des Membres dans la mise en œuvre de ces normes et dans la mise en place de compartiments sera bénéfique à la Commission dans le cadre de la révision de ce chapitre. Les Membres sont invités à faire part de leur expérience en matière de mise en œuvre des normes relatives à la compartimentation du *Code aquatique*. À toutes fins utiles, plusieurs questions sont adressées aux Membres afin qu'ils disposent d'une trame pour formuler leurs réponses.

Questions aux Membres :

1. L'Autorité compétente et les Services en charge de la santé des animaux aquatiques dans votre pays ont-ils établi des compartiments ? Si tel est le cas, quel est l'objectif de la mise en place de ces compartiments (par exemple, création d'écloserie, production d'animaux aquatiques ou fabrication de produits qui en sont issus, qu'ils soient destinés à la consommation humaine, aux échanges commerciaux nationaux ou internationaux).

2. Quelle est le retour d'expérience de votre pays dans la mise en place de compartiments, et notamment sur les points suivants :

    a) un retour d'expérience positif (décrire par exemple les bénéfices retirés en matière de gestion de la santé des animaux aquatiques ou de leur commercialisation) ;

    b) des obstacles rencontrés dans la mise en place des compartiments ;

    c) l'utilité des normes relatives à la compartimentation (chapitres 4.2. et 4.3.) du C*ode aquatique* (par exemple, l'information utile, les lacunes, la nécessité d'accorder une importance à certains points ou de disposer d'orientations).

    d) l'acceptation des compartiments établis par les partenaires commerciaux internationaux ?

3. Avez-vous développé au niveau national des politiques ou procédures en matière de compartimentation ? Si tel est le cas, accepteriez-vous de les porter à la connaissance de la Commission des animaux aquatiques afin qu'elle les prenne en considération lorsqu'elle révisera le chapitre 4.3. ?

4. Avez-vous consulté les acteurs de votre industrie sur l'intérêt qu'ils portent à la mise en place de compartiments? Si tel est le cas, quels secteurs de l'industrie ont exprimé un intérêt ? Quel est l'objectif de la mise en place de tels compartiments ?

_____

SECTION 2.2.

DISEASES OF CRUSTACEANS

CHAPTER 2.2.0.

GENERAL INFORMATION

A. SAMPLING

1. Assessing the health status of the epidemiological unit

1.1. Sample material to be used for tests

Sample material and the number of samples to be collected depend on the specific disease or pathogen, the size of animals and the objective of testing (i.e. diagnosis of overt disease, detection of subclinical infection in apparently healthy animals or sampling for targeted surveillance to demonstrate freedom from infection with a specified pathogen). See the individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

1.2. Specifications according to crustacean populations

For details of animals to sample for a specific listed disease, see the relevant disease chapter in the *Aquatic Manual*. The design of a surveillance system for demonstrating disease-free status for a country, zone or compartment should be in accordance with the recommendations of the OIE *Aquatic Code* Chapter 1.4.

Animals to be sampled are selected as follows:

i) Susceptible species should be sampled proportionately or following risk-based criteria for targeted selection of lots or populations with a history of abnormal mortality or potential exposure events (e.g. replacement with stocks of unknown disease status).

ii) If more than one water source is used for production, animals from all water sources should be included in the sample.

iii) For the study of presumptively diseased crustaceans select those animals that are moribund, discoloured, displaying abnormal behaviour, or otherwise abnormal.

iv) When sampling is aimed at assessing disease occurrence (e.g. estimation of disease prevalence), the preferred selection method is probability sampling.

1.3. Specifications according to clinical status

In clinical disease episodes, carefully selected quality specimens with representative lesions should be obtained from live or moribund crustaceans. Collection of dead specimens during disease outbreaks should be avoided when possible, but recently dead samples may be suitable for some diagnostic assays. When cultured or wild crustacean stocks are presenting clinical signs of an active disease that are consistent with, or suggestive of, any one of the OIE-listed crustacean diseases, care should be taken to ensure that the samples collected are preserved appropriately for the anticipated diagnostic tests (see sample preservation section for recommended methods). In situations other than when clinical disease episodes are investigated, for the OIE-listed diseases it is highly recommended that the scheduling of sampling be planned (i.e. by farm schedule, season, etc.) so that the particular life-stage(s) are sampled at a time when the pathogen of concern is most likely to be detected. Disease-specific

recommendations are provided in Section 3 *Sample selection, sample collection, transportation and handling* of the individual chapters.

Recently dead crustaceans may be suitable (depending on their condition) for certain diagnostic assays such as nucleic acid detection techniques.

1.4. Specifications according to crustacean size

See the individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

2. General processing of samples

2.1. Macroscopic examination

See disease-specific chapters in this *Aquatic Manual*.

2.2. Virological examination

2.2.1. Transportation and antibiotic treatment of samples

Culture systems for crustacean viruses are not available; antibiotic treatment of samples is not required. For transportation of samples see Section 3 of disease-specific chapters in this *Aquatic Manual*.

2.2.2. Virus isolation

For processing of tissues see Section 3 of disease-specific chapters in this *Aquatic Manual*.

2.2.3. Treatment to neutralise enzootic viruses

Not applicable.

2.3. Bacteriological examination

The strains of *Vibrio parahaemolyticus* (Vp_{AHPND}) that cause acute hepatopancreatic necrosis disease (AHPND) can be isolated on standard bacteriological media. *Hepatobacter penaei*, the causative agent of necrotising hepatopancreatitis (NHP) has not been cultured and, because of its very small size, bacteriological examination may be limited to Gram staining. See disease-specific chapters in this *Aquatic Manual* for identification methods.

2.4. Parasitic examination

Not applicable for currently listed diseases.

2.5. Fungal and other protists examination

See Chapter 2.2.2 *Infection with* Aphanomyces astaci (*Crayfish plague*).

B. MATERIALS AND BIOLOGICAL PRODUCTS REQUIRED FOR THE ISOLATION AND IDENTIFICATION OF CRUSTACEAN PATHOGENS

1. Crustacean viruses

1.1. Crustacean cell lines

Not applicable. There are currently no confirmed or documented crustacean cell lines.

1.2. Culture media

Not applicable.

1.3. Virus positive controls and antigen preparation

1.3.1. Virus nomenclature

In general, the virus nomenclature used in the disease-specific chapters follows the most recent taxonomy for viruses as given in the Report of the Committee on Taxonomy of Viruses (see: ICTV [ictvonline.org] for latest information). Also provided in the disease-specific chapters are the disease and virus names that are in common use by the shrimp/prawn farming industries, as well as the more common synonyms that have been used or are in current use.

1.3.2. Virus production

As no cell lines (crustacean, arthropod, or vertebrate) are known that can be used to produce crustacean viruses, infection of known susceptible host species (which are free of infection by the agent in question) is the preferred method for virus production for experimental purposes.

1.3.3. Virus preservation and storage

Infectivity of all of the OIE-listed crustacean viruses can be preserved by freezing infected whole crustaceans or infected target tissues at –20°C for short-term storage, or at –80°C or lower for long-term storage.

2. Crustacean bacteria

2.1. Culture media

See Chapter 2.2.1. Acute hepatopancreatic necrosis disease for details.

2.2. Storage of cultures

Lyophilisation or storage at –70°C is recommended for long-term storage of bacterial cultures.

3. Crustacean parasites

3.1. Culture media

Not applicable for currently listed diseases.

3.2. Storage of cultures

Not applicable for currently listed diseases.

4. Crustacean fungi and protists

4.1. Culture media

See chapter 2.2.2.

4.2. Storage of cultures

See chapter 2.2.2.

5. Techniques

The available diagnostic methods that may be selected for diagnosis of the OIE-listed crustacean diseases or detection of their aetiological agents are based on:

i) Gross and clinical signs.

ii) Direct bright-field, phase-contrast or dark-field microscopy with whole stained or unstained tissue wet-mounts, tissue squashes, and impression smears; and wet-mounts of faecal strands.

iii) Histology of fixed specimens.

iv) Bioassays of suspect or subclinical carriers using a highly susceptible host (life stage or species) as the indicator for the presence of the pathogen.

v) Antibody-based tests for pathogen detection using specific antisera, polyclonal antibodies (PAbs) or monoclonal antibodies (MAbs).

vi) Molecular methods (including sequencing):

DNA probes or RNA probes for *in-situ* hybridisation (ISH) assays with histological sections of fixed tissues;

Conventional and real-time PCR/RT-PCR and LAMP for direct assay with fresh tissue samples or with extracted DNA or RNA.

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger crustaceans should be processed and tested individually. However, for eggs, larvae and postlarvae pooling of larger numbers (e.g. ~150 or more eggs or larvae or 50 to 150 postlarvae depending on their size/age) may be necessary to obtain sufficient sample material to run a diagnostic assay.

5.1. Antibody-based tests

See disease-specific chapters in this *Aquatic Manual*.

5.2. Direct microscopy

Samples for direct microscopic examination should be examined as soon as possible after collection. Use live specimens whenever possible, or use fresh, chilled, or fixed specimens when live specimens are not practical. If an adequate field laboratory is available, it should be used to process and examine samples near the site of collection.

5.3. Histological techniques

Only live or moribund specimens with clinical signs should be sampled for histology. Collect crustaceans by whatever means are available with a minimum of handling stress. Hold animals in a container appropriate for maintaining suitable water quality and supply adequate aeration to the container if the crustaceans are to be held for a short period of time before actual fixation.

5.3.1. Fixation

A general rule is that a minimum of ten volumes of fixative should be used for one volume of tissue sample (i.e. a 10 g sample of crustacean would require 100 ml of fixative).

i) Davidson's AFA (alcohol, formalin, acetic acid) fixative

Davidson's AFA fixative is recommended for most histological applications. The fixative is rapid, reduces autolytic changes in decapod crustaceans (i.e. especially in crustaceans in tropical and subtropical regions), and its acidic content decalcifies the cuticle. The formulation for Davidson's AFA is (for 1 litre):

330 ml 95% ethyl alcohol
220 ml 100% freshly made formalin (a saturated 37–39% aqueous solution of formaldehyde gas)

4. 115 ml glacial acetic acid

335 ml tap water (for marine crustaceans, seawater may be substituted)
Store the fixative in glass or plastic bottles with secure caps at room temperature.

ii) Fixation procedures with Davidson's AFA

For larvae and postlarvae that are too small to be easily injected with fixative using a tuberculin syringe: Using a fine mesh screen or a Pasteur pipette, select and collect specimens. Immerse crustaceans selected for sampling directly in the fixative. Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

For juveniles that are too small to be injected: Select and collect specimens. Use a needle or fine-pointed forceps to incise the cuticle and immediately immerse crustaceans selected for sampling directly into the fixative. Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

For large juveniles and adults: to ensure proper fixation, kill the crustacean using a humane method, then immediately inject fixative (use 5–10% volume:weight). Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

The hepatopancreas (HP) should be injected first and at two or more sites, with a volume of fixative sufficient to change the HP to a white-to-orange colour (when Davidson's AFA is used); then inject fixative into adjacent regions of the cephalothorax, into the anterior abdominal region, and into the posterior abdominal region.

The fixative should be divided between the different regions, with the cephalothoracic region, specifically the HP, receiving a larger share than the abdominal region.

Immediately following injection, slit the cuticle with dissecting scissors, from the sixth abdominal segment to the base of the rostrum, being particularly careful not to cut deeply into the underlying tissue. The incision in the cephalothoracic region should be just lateral to the dorsal midline, while that in the abdominal region should be approximately mid-lateral.

For crustaceans larger than ~12 g: After injection of fixative, the body should then be transversely bisected, at least once, just posterior to the abdomen/cephalothorax junction, and (optional) again mid-abdominally.

For very large crustaceans (*e.g. lobsters, crabs, adult penaeids, adult Macrobrachium rosenbergii, some species and life stages of crabs, crayfish, etc.*): The organs of interest may be excised after injection of fixative. Completion of fixation of these tissue samples is then handled as outlined previously.

Following injection, incisions and bisection/trisection, or excision of key organs, immerse the specimen in the fixative (use 10:1 fixative:tissue ratio).

Allow fixation to proceed at room temperature for 24–72 hours depending on the size of crustacean being preserved. Longer fixation times in Davidson's AFA may be used to thoroughly decalcify the shell of crabs, lobsters, crayfish, etc.

Following fixation, the specimens should be transferred to 70% ethyl alcohol, where they can be stored for an indefinite period.

iii) Transport and shipment of preserved samples

As large volumes of alcohol should not be mailed or shipped, the following methods are recommended: Remove the specimens from the 70% ethyl alcohol. For larvae, postlarvae, or small juveniles, use leak-proof, screw-cap plastic vials if available; if glass vials must be used, pack to prevent breakage. For larger specimens, wrap samples with white paper towels to completely cover (do not use raw or processed cotton). Place towel-wrapped specimens in a sealable plastic bag and saturate with 70% ethyl alcohol. Insert the label and seal the bag. Place the bag within a second sealable bag. Multiple small sealable bags can again be placed within a sturdy, crush-proof appropriately labelled container for shipment (for details see *Aquatic Code* Chapter 5.10 *Measures concerning international transport of aquatic animal pathogens and pathological material*).

5.4. Transmission or scanning electron microscopy

Electron microscopy (EM – transmission or scanning) is a valuable research tool for the study of disease in crustaceans. However, EM methods are not routinely used for diagnosis of the diseases listed by the OIE.

5.5. Use of molecular and antibody-based techniques for confirmatory testing and diagnosis

Molecular techniques, including the use of nucleic acid probes for *in-situ* hybridisation, conventional PCR and real-time PCR, have been developed for the identification of many pathogens of aquatic animals. Real-time PCR methods, in general, have high sensitivity and specificity and, following adequate validation, can be used for direct detection of viral nucleic acids in samples prepared from crustacean tissue. The technique can be used in direct surveillance of apparently healthy populations, if they have a high level of diagnostic sensitivity, as well as in the diagnosis of clinically affected animals.

When using PCR as a diagnostic method, the design of primers and probe, the use of positive and negative controls, as well as validation of the PCR method chosen are important. Real-time PCR is a powerful technique particularly for analysing relatively high numbers of samples (e.g. for surveillance) via high-throughput testing. Several nucleic acid probe and PCR protocols are included in this version of the *Aquatic Manual* as screening, diagnostic or confirmatory methods for crustaceans and can be undertaken as the standard method. Following real-time PCR-positive results, where possible, conventional PCR with sequencing of PCR products should be used for confirmation of pathogen identity.

As with all PCR protocols, optimisation may be necessary depending on the reagents, equipment and the plasticware used. PCR is prone to false-positive and false-negative results. False-positive results (negative samples giving a positive reaction) may arise from either product carryover from positive samples or, more commonly, from cross-contamination by PCR products from previous tests. False-negative results (positive samples giving a negative result) may lead to unwanted transmission of pathogens and biosecurity failure.

Diagnostic samples should be tested in duplicate and both must produce positive results for a sample to be deemed positive. In instances where a sample produces one positive and one negative result, these are deemed indeterminate and should be retested. In addition, the following controls should be run with each assay: negative extraction control (e.g. a tissue [or equivalent sample that is under test]) sample from a known uninfected animal; positive control (preferably, one that can be distinguished from the pathogen genomic sequence [e.g. an artificial plasmid], thus allowing detection of any cross-contamination leading to a false positive result); no template control (all reagents with water replacing the template); internal positive control (internal housekeeping gene). All controls should produce their expected results in order for the diagnostic test result to be valid.

To minimise the risk of contamination, aerosol-preventing pipette tips should be used for all sample and PCR preparation steps. Additionally, all PCRs should be prepared in a clean area that is separate from the area where the amplifications and gel electrophoresis are performed. Do not share equipment (e.g. laboratory coats and consumables) between areas and, where possible, restrict access between areas. Contaminating PCR products can be carried on equipment, clothes, shoes and paper (e.g. workbooks). Also, ensure all work-tops and air-flow hoods used for the extractions and PCR set-up are regularly cleaned and decontaminated. To ensure sample integrity, always store the samples (e.g. in a freezer or refrigerator) in a location away from the molecular biology laboratory and reagents.

5.5.1. Sample preparation and types

Samples selected for nucleic acid-based or antibody-based diagnostic tests should be handled and packaged (in new plastic sample bags or bottles) with care to minimise the potential for cross-contamination among the sample set taken from different (wild or farmed) stocks, tanks, ponds, farms, etc. A water-resistant label, with the appropriate data filled out, should be placed within each package or container for each sample set.

Some suitable methods for preservation and transport of samples taken for molecular or antibody-based tests are:

i) *Live specimens:* these may be processed in the field or shipped to the diagnostic laboratory for testing.

ii) *Haemolymph:* this tissue is the preferred sample for certain molecular and antibody-based diagnostic tests (see disease-specific chapters). Samples may be collected by needle and syringe through cardiac puncture, from the haemocoel (i.e. the ventral sinus in penaeids), or from a severed appendage, and immediately transferred to a tube that is half full with 90–95% ethanol or suitable nucleic acid preservative.

iii) *Iced or chilled specimens:* these are specimens that can be transported to the laboratory for testing within 24 hours. Pack samples in sample bags surrounded by an adequate quantity of wet ice around the bagged samples in an insulated box and ship to the laboratory.

iv) *Frozen whole specimens:* select live specimens according to the criteria listed in disease-specific chapters in this *Aquatic Manual*, quick freeze in the field using crushed dry-ice or freeze in the field laboratories using a mechanical freezer at –20°C or lower temperature. Prepare and insert the label into the container with the samples, pack samples with an adequate quantity of dry-ice in an insulated box, and ship to the laboratory.

v) *Alcohol-preserved samples:* in regions where the storage and shipment of frozen samples is problematic, 90–95% ethanol may be used to preserve, store, and transport certain types of samples for molecular tests. Alcohol-preserved samples are generally not suitable for antibody-based tests. Whole crustaceans (any life stage provided the specimen is no larger than 2–3 g), excised tissues (i.e. pleopods) from large crustaceans, or haemolymph may be preserved in 90–95% ethanol, and then packed for shipment according to the methods described in Section 5.3.1, paragraph iii (see chapter 5.10 of the *Aquatic Code* for additional details on the international transport of such samples).

5.5.2. Preservation of RNA and DNA in tissues

For routine diagnostic testing by PCR or RT-PCR, samples must be prepared to preserve the pathogen's nucleic acid. For most purposes, preservation of samples in alcohol (80–90%) is the preferred method for subsequent molecular tests. Samples preserved in this way can be stored at 4°C for 1 month, at 25°C for 1 week or indefinitely at –20°C or below. In addition, other products (e.g. nucleic acid preservatives, various lysis buffers, etc.) are commercially available for the same purpose.

5.5.3. Nucleic acid extraction

To isolate nucleic acids from tissues preserved in ethanol or nucleic acid preservative, simply remove the tissue from the fixative or preservative and treat it as though it was just harvested. Most fresh and preserved or fixed tissues can be homogenised (e.g. with a mortar and pestle or in bead-beating tubes) directly in the lysis or extraction buffer provided with commercially available DNA and RNA extraction kits. Commercial kits should be validated or undergo equivalence testing with current validated extraction procedures prior to routine use.

5.5.4. Preparation of slides for *in-situ* hybridisation

For *in-situ* hybridisation, fixed tissues that have been transferred to 70% ethanol are embedded in paraffin according to standard histological methods. Sections are cut at a thickness of 5 μm and placed on aminoalkylsilane-coated slides, which are then baked overnight in an oven at 40°C. The sections are de-waxed by immersing in xylene for 10 minutes. This step is repeated once and then the solvent is eliminated by immersion in two successive absolute ethanol baths for 10 minutes each. The sections are then rehydrated by immersion in an ethanol series. The protocol may require a step of membrane permeabilisation enabling access to the target DNA. For this purpose, sections are treated with proteinase K (100 μg ml^{-1}) in TE buffer (Tris [50 mM], EDTA [10 mM]), at 37°C for 30 minutes. For in-situ hybridisation tests (see individual chapters for details), it is essential that both a known positive and a known negative slide be stained to eliminate false positive results due to non-specific staining/stain dropout, and false negative results due to errors in the staining protocol (Qadiri *et al*., 2019; Valverde *et al*., 2017).

For further details see disease-specific chapters in this *Aquatic Manual*.

6. Additional information to be collected

Sample information should include the collector's name, organisation, date, time, and description of the geographical location. The geographical origin of samples may be described as the name or location of the sampling site or its geographical co-ordinates. There should also be records that provide information to allow trace-backs on the sample movement from the sample site to the storage facility or laboratory and within those facilities.

A history of the specimens should also be collected and should include species, age, weight, details of clinical signs including behavioural changes, as well as observations concerning any gross pathology which has been observed.

Information on the preservation method, storage location, and date and time of storage at each storage locker or freezer along with information on the storage temperature (continuously monitored is preferable) should be collected. This information should be tracked with a unique sample code for all samples. For laboratories, the date of receipt, storage location information, date of analysis, analysis notes, and report date should be maintained for all uniquely coded samples.

These data will greatly facilitate the tracking of sample problems and provide assurance that the samples were properly handled.

5. KEY REFERENCES FOR FURTHER READING

BELL T.A. & LIGHTNER D.V. (1988). A Handbook of Normal Shrimp Histology. Special Publication No. 1, World Aquaculture Society, Baton Rouge, Louisiana, USA.

BONDAD-REANTASO M.G., MCGLADDERY S.E., EAST I. & SUBASINGHE R.P. (2001). Asian Diagnostic Guide to Aquatic Animal Diseases. *FAO Fisheries Technical Paper*, No. 402, supplement 2. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 240 pp.

JOHNSON P.T. (1980). Histology of the Blue Crab, *Callinectes sapidus*. A Model for the Decapoda. Prager, New York, USA, 440 pp.

LIGHTNER D.V. (1996). A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA. 304 pp.

LIGHTNER D.V., REDMAN R.M., PANTOJA C.R., TANG K.F.J, NOBLE B.L., SCHOFIELD P., MOHNEY L.L., NUNAN L.M. & NAVARRO S.A. (2012). Historic emergence, impact and current status of shrimp pathogens in the Americas. *J. Invert. Pathol.,* **110**, 174–183.

LOTZ J.M. (1997). Special topic review: Viruses, biosecurity and specific pathogen-free stocks in shrimp aquaculture. *World J. Microbiol. Biotechnol*., **13**, 405–413.

MOODY N.J.G. & CRANE M.ST.J. (2016). Validation of diagnostic tests in the OIE manual for aquatic animals. In: Proc. 3rd OIE Global Conference on Aquatic Animal Health – "Riding the Wave of the Future", Ho Chi Minh City, Vietnam, 20–22 January 2015, pp.119–126.

QADIRI S.S.N., SOO-JIN KIM S.-J., KRISHNAN R., KIM J.-O., KOLE S., KIM W.-S. & OH M.-J. (2019). Localization and tissue tropism of viral haemorrhagic septicemia virus (VHSV) in experimentally infected juvenile olive flounder, *Paralichthys olivaceus*: An *in situ* hybridization and immunohistochemical study. *Aquaculture*, **505**, 242–252.

THITAMADEE S, PRACHUMWAT A., SRISALA J., JAROENLAK P., SALACHAN P.V., SRITUNYALUCKSANA K, FLEGEL T.W. & ITSATHITPHAISARN O. (2016). Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, **452**, 69–87.

VALVERDE E.J., BORREGO J.J., SARASQUETE M.C., ORTIZ-DELGADO J.B. & CASTRO D. (2017). Target organs for lymphocystis disease virus replication in gilthead seabream (*Sparus aurata*). *Vet. Res*., **48**, 21. doi 10.1186/s13567- 017-0428-3.

WALKER P.J. & MOHAN C.V. (2009). Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies. *Rev. Aquaculture*, **1**, 125–154.

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NB: FIRST ADOPTED IN 2000; MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.2.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

1. Scope

Acute hepatopancreatic necrosis disease (AHPND) means infection with strains of *Vibrio parahaemolyticus* (Vp_{AHPND}) that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB. Although there are reports of the isolation of other *Vibrio* species from clinical cases of AHPND, only Vp_{AHPND} has been demonstrated to cause AHPND.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

AHPND has a bacterial aetiology (Kondo *et al*., 2015; Tran *et al*., 2013). It is caused by specific virulent strains of *V. parahaemolyticus* (Vp_{AHPND}) that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) binary toxin, PirA and PirB (Gomez-Gil *et al.,* 2014; Gomez-Jimenez *et al*., 2014; Han *et al.,* 2015a; Kondo *et al.,* 2014; Lee *et al.,* 2015; Yang *et al.,* 2014). The plasmid within Vp_{AHPND} has been designated pVA1, and its size may vary slightly. Removal (or "curing") of pVA1 abolishes the AHPND-causing ability of Vp_{AHPND} strains.

Within a population of Vp_{AHPND} bacteria, natural deletion of the Pir^{vp} operon may occur in a few individuals (Lee *et al.,* 2015; Tinwongger *et al.,* 2014). This deletion is due to the instability caused by the repeat sequences or transposase that flank the Pir toxin operon. When the deletion occurs, it means that a Vp_{AHPND} strain will lose its ability to induce AHPND. However, if the Pir toxin sequence is used as a target for detection, then a colony that has this deletion will produce a negative result even though the colony was derived from an isolate of AHPND-causing Vp_{AHPND}. A recent report describes a naturally occurring deletion mutant of Vp_{AHPND} that does not cause a clinical manifestation of AHPND (Aranguren *et al.,* 2020a).

The plasmid pVA1 also carries a cluster of genes related to conjugative transfer, which means that this plasmid is potentially able to transfer to other bacteria.

2.1.2. Survival and stability in processed or stored samples

AHPND cannot be transmitted from infected samples that have been stored frozen (Tran *et al*., 2013). Some *Vibrio* species are sensitive to freezing (Muntada-Garriga *et al*., 1995; Thomson & Thacker, 1973).

2.1.3. Survival and stability outside the host

Vp_{AHPND} is expected to possess similar properties to other strains of *V. parahaemolyticus* found in seafood that have been shown to survive up to 9 and 18 days in filtered estuarine water and filtered seawater at an ambient temperature of 28 ± 2°C (Karunasagar *et al*., 1987).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to AHPND according to Chapter 1.5. of the *Aquatic Code* are: giant tiger prawn (*Penaeus monodon*) and whiteleg shrimp (*Penaeus vannamei*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to AHPND according to Chapter 1.5. of the *Aquatic Code* are: fleshy prawn (*Penaeus chinensis*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: kuruma prawn (*Penaeus japonicus*).

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Mortalities occur within 30–35 days, and as early as 10 days, of stocking shrimp ponds with postlarvae (PL) or juveniles (Joshi *et al*., 2014b; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013). De la Pena *et al*. (2015) reported disease outbreaks in the Philippines occurring as late as 46–96 days after pond-stocking.

2.2.4. Distribution of the pathogen in the host

Gut including stomach, and hepatopancreas.

2.2.5. Aquatic animal reservoirs of infection

~~In experimental challenges, *Macrobrachium rosenbergii* and *Cherax quadricarinatus* did not show clinical signs of the disease or histopathological changes induced by AHPND but tested positive by PCR assay. However, whether these species serve as reservoirs of infection or are resistant to AHPND needs further investigation (Powers *et al.,* 2021; Schofield *et al.,* 2020).~~ None known.

2.2.6. Vectors

No vector is known, although as *Vibrio* spp. are ubiquitous in the marine environment, the possibility that there are vector species could be expected.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

AHPND is characterised by sudden, mass mortalities (up to 100%) usually within 30–35 days of stocking grow-out ponds with PLs or juveniles (Hong *et al.,* 2016). Older juveniles may also be affected (de la Pena *et al*., 2015).

In regions where AHPND is enzootic in farmed shrimp, evidence indicates a near 100% prevalence (Tran *et al*., 2014).

2.3.2. Clinical signs, including behavioural changes

The onset of clinical signs and mortality can start as early as 10 days post-stocking. Clinical signs include: moribund prawns sink to bottom, pale to white hepatopancreas (HP) due to pigment loss in the connective tissue capsule (NACA, 2014). ~~Clinical signs include a pale-to-white hepatopancreas (HP), significant atrophy of the HP, soft shells, guts with discontinuous, or no contents and black spots or streaks visible within the HP (due to melanised tubules). In addition, the HP does not squash easily between the thumb and forefinger (probably due to increased fibrous connective tissue and haemocytes) (NACA, 2014).~~

2.3.3 Gross pathology

Gross pathological observations include pale-to-white HP, significant atrophy of the HP, soft shells, guts with discontinuous, or no contents and black spots or streaks visible within the HP (due to melanised tubules). In addition, the HP does not squash easily between the thumb and forefinger (probably due to increased fibrous connective tissue and haemocytes) (NACA, 2014). ~~AHPND has three infection phases. In the acute phase, there is massive and progressive degeneration of the HP tubules from proximal to distal, with significant rounding and sloughing of the HP tubule epithelial cells into the lumen of the tubule, the HP collecting ducts and the posterior stomach and the absence of bacterial cells. In the terminal phase, the HP shows intra-tubular haemocytic inflammation and develops massive secondary bacterial infections that occur in association with~~

~~the necrotic and sloughed HP tubule cells. Animals that survive an acute infection reach a chronic phase, in which they present with limited cellular changes in the hepatopancreas tubule and only a few tubules with epithelial necrosis accompanied by bacteria and inflammation. The chronic phase pathology resembles a septic hepatopancreatic necrosis (SHPN) (Aranguren *et al.*, 2020a; NACA, 2014; Nunan *et al.*, 2014; Soto-Rodriguez *et al.*, 2015; Tran *et al.*, 2013; 2014).~~

2.3.4. Modes of transmission and life cycle

Vp$_{AHPND}$ has been transmitted experimentally by immersion, feeding (*per os*) and reverse gavage (Dabu *et al.,* 2017; Joshi *et al*., 2014b; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013), simulating natural horizontal transmission via oral routes and co-habitation.

2.3.5. Environmental factors

Water sources with low salinity (<20 ppt) seem to reduce the incidence of the disease. Peak occurrence seems to occur during the hot, dry season from April to July. Overfeeding, poor seed quality, poor water quality, poor feed quality, algal blooms or crashes are also factors that may lead to occurrences of AHPND in endemic areas (NACA, 2014).

2.3.6. Geographical distribution

The disease was initially reported in Asia in 2010. It has since been reported in the Americas (2013) and Africa (2017).

See OIE WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Not available.

2.4.2. Chemotherapy including blocking agents

Not available.

2.4.3. Immunostimulation

None known to be effective.

2.4.4. Breeding resistant strains

Not available.

2.4.5. Inactivation methods

Experimental studies have shown that *Vp*$_{AHPND}$ could not be transmitted via frozen infected shrimp (Tran *et al*., 2013). Similarly, other strains of *V. parahaemolyticus* are known to be sensitive to freezing, refrigeration, heating and common disinfectants (Muntada-Garriga *et al*., 1995; Thomson & Thacker, 1973).

2.4.6. Disinfection of eggs and larvae

Not available.

2.4.7. General husbandry

As with other infectious diseases of shrimp, established good sanitary and biosecurity practices, such as improvement of hatchery sanitary conditions and PL screening are likely to be beneficial; good broodstock management, use of high-quality post-larvae and good shrimp farm management including strict feeding rate control, appropriate stocking density etc. are all well-established practices that reduce the impact of disease, including AHPND. An AHPND-tolerant line of *P. vannamei* was recently reported, but at present (2022) no genetically improved lines are commercially available (Aranguren *et al.,* 2020b).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Samples of moribund shrimp or shrimp that show clinical signs (see Section 2.3.2) should be selected for AHPND diagnosis. It is assumed that adults (broodstock) can carry strains of *Vp*$_{AHPND}$ (Lee *et al*., 2015; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013). Therefore, broodstock without clinical signs may also be selected for diagnostic testing.

3.2. Selection of organs or tissues

Samples may be taken from gut-associated tissues and organs, such as the hepatopancreas, stomach, midgut and hindgut. ~~In the case of valuable broodstock, non-lethal faecal samples may be collected instead, however the utility of faecal samples compared with tissue samples has not been evaluated.~~

3.3. Samples or tissues not suitable for pathogen detection

Samples other than gut-associated tissues and organs are not appropriate (NACA, 2014; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013).

3.4. Non-lethal sampling

Faecal matter may be collected from valuable broodstock for AHPND diagnosis. However, compared with tissue sampling, the relative utility of faecal samples for detecting AHPND-causing bacteria has not been evaluated.

3.5. Preservation of samples for submission

~~Samples to be submitted are (i) fresh and chilled on ice for bacterial isolation, (ii) fixed in 90% ethanol for PCR detection and (iii) preserved in Davidson's AFA fixative for histology (Joshi *et al.,* 2014a; 2014b; Lee *et al.,* 2015; Nunan *et al.,* 2104; Sirikharin *et al.,* 2015; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013).~~

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0.

3.5.1. Samples for pathogen isolation

High quality samples are essential for successful pathogen isolation and bioassay. Sample quality depends mainly on the time since collection and time spent in storage. Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animals and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. Alternatively, samples can be preserved in DNAzol for PCR testing. If material cannot be fixed it may be frozen, but repeated freezing and thawing of samples should be avoided.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology, immunohistochemistry or in-situ hybridization can be preserved in Davidson's AFA fixative for histology (Joshi *et al.,* 2014a; 2014b; Nunan *et al.,* 2014; Sirikharin *et al.,* 2015; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013).

3.5.4. Samples for other tests

Not applicable.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger specimens should be processed and tested individually. Small life stages can be pooled to obtain the minimum amount of material for bacterial isolation or molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology		+	+	NA		+	+	NA				
Cell culture												
Real-time PCR	++	++	++	1	++	++	++	1	++	++	++	1
Conventional PCR	++	++	++	2	++	++	++	2	~~++~~	~~++~~	~~++~~	~~2~~
Conventional PCR followed by amplicon sequencing									+++	+++	+++	~~1~~2
In-situ hybridisation												
Bioassay					+	+	+	NA	+	+	+	NA
LAMP		++	++	1								
Ab-ELISA												
Ag-ELISA		±	++	1		±	++	1		±	++	1
Other antigen detection methods[3]												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; NA = Not available.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

The disease has three distinct phases:

i) The acute phase is characterised by a massive and progressive degeneration of the HP tubules from proximal to distal, with significant rounding and sloughing of HP tubule epithelial cells into the HP tubules, HP collecting ducts and posterior stomach in the absence of bacterial cells (Nunan *et al*., 2014; Soto-Rodriguez *et al*., 2015; Tran *et al*., 2013; 2014).

ii) The terminal phase is characterised by marked intra-tubular haemocytic inflammation and development of massive secondary bacterial infections that occur in association with the necrotic and sloughed HP tubule cells (NACA, 2012; Nunan *et al*., 2014; Soto-Rodriguez *et al*., 2015; Tran *et al*., 2013; 2014).

iii) The chronic phase is characterised by only a few tubules with epithelial necrosis accompanied by bacteria and inflammation. This phase resembles a septic hepatopancreatic necrosis (SHPN) (Aranguren *et al*., 2020b).

4.3. Cell culture for isolation

4.3.1. Enrichment of samples prior to DNA extraction

Preliminary enrichment culture for detection of *Vp*$_{AHPND}$ from sub-clinical infections or environmental samples may be carried out using any suitable bacteriological medium (e.g. tryptic–soy broth or alkaline peptone water containing 2.5% NaCl supplement) incubated for 4 hours at 30°C with shaking. Then, after letting any debris settle, the bacteria in the culture broth are pelleted by centrifugation. Discarding the supernatant, DNA can be extracted from the bacterial pellet in preparation for PCR analysis.

4.3.2. Agent purification

Vp$_{AHPND}$ may be isolated in pure culture from diseased shrimp, sub-clinically infected shrimp, or environmental samples using standard microbiological media for isolation of *Vibrio* species from such sources (Lightner, 1996; Tran *et al*., 2013). Confirmation of identification of *Vp*$_{AHPND}$ may be undertaken by PCR analysis.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

PCR methods have been developed that target the Vp$_{AHPND}$ toxin genes. The AP3 method is a single-step PCR that targets the 12.7 kDa PirA^{vp} gene (Sirikharin *et al*., 2015). It was validated for ~~100% positive and negative predictive value by testing 104~~ isolates of Vp$_{AHPND}$ and non-pathogenic bacteria (including other *Vibrio* and non-*Vibrio* species) that had previously been tested by bioassay (Sirikharin *et al*., 2015). Subsequently, Soto-Rodriguez *et al*. (2015), using 9 Vp$_{AHPND}$ and 11 non-pathogenic isolates of *V. parahaemolyticus* reported that the AP3 method produced the highest positive (90%) and negative (100%) predictive values of five PCR methods tested.

Single-step PCRs such as the AP3 method and others, e.g. VpPirA-284, VpPirB-392 (Han *et al*., 2015a) and TUMSAT-Vp3 (Tinwongger *et al*., 2014), have relatively low sensitivity when used for detection of Vp$_{AHPND}$ at low levels (e.g. sub-clinical infections~~) or in environmental samples such as sediments and biofilms~~. For such samples, a preliminary enrichment step (see Section 4.3.1. *Enrichment of samples prior to DNA extraction*) is recommended.

Alternatively, a nested PCR method, AP4, has been developed with a 100% positive predictive value for Vp$_{AHPND}$ using the same 104 bacterial isolates used to validate AP3 above (Dangtip *et al*., 2015), and has greater sensitivity (1 fg of DNA

extracted from Vp_{AHPND}), allowing it to be used directly with tissue ~~and environmental samples~~ without an enrichment step.

In addition, real-time PCR methods, for example the Vp_{AHPND}-specific TaqMan real-time PCR developed by Han *et al*. (2015b), and an isothermal loop-mediated amplification protocol (LAMP) method developed by Koiwai *et al*. (2016) also have high sensitivity and can be used directly with tissue ~~and environmental samples~~ without an enrichment step.

A general DNA extraction method may be used to extract DNA from the stomach or hepatopancreatic tissue of putatively infected shrimp, from cultures of purified bacterial isolates or from bacterial pellets from enrichment cultures (see Section 4.3). The amount of template DNA in a 25 µl PCR reaction volume should be in the range of 0.01–1 ng of DNA when extracted from bacterial isolates (i.e. directly from a purified culture) and in the range of 10–100 ng of total DNA when extracted from shrimp tissues or from a bacterial pellet derived from an enrichment culture.

~~The following controls should be included in all *Vp*$_{AHPND}$ PCR assays: a) negative extraction control i.e. DNA template extracted at the same time from a known negative sample; b) DNA template from a known positive sample, such as *Vp*$_{AHPND}$-affected shrimp tissue or DNA from an *Vp*$_{AHPND}$-positive bacterial culture, or plasmid DNA that contains the target region of the specific set of primers; c) a non-template control. In addition, a further control is required to demonstrate that extracted nucleic acid is free from PCR inhibitors, for example, for shrimp tissues use of the decapod 18S rRNA PCR (Lo *et al*., 1996) or use the 16S rRNA PCR for bacteria (Weisburg *et al*., 1991).~~

4.4.1. Real-time PCR

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Han *et al*., 2015b; GenBank KM067908			
pirA	Fwd VpPirA-F: TTG-GAC-TGT-CGA-ACC-AAA-CG Rev VpPirA-R: GCA-CCC-CAT-TGG-TAT-TGA-ATG VpPirA Probe: FAM-AGA-CAG-CAA-ACA-TAC-ACC-TAT-CAT-CCC-GGA-TAMRA	Fwd: 0.3 µM Rev: 0.3 µM probe: 0.1 µM	95°C/20 sec; 45 cycles 95°C/3 sec and 60°C/30 sec

~~This protocol is based on the method described by Han *et al*. (2015b). The TaqMan Fast Universal PCR Master Mix (Life Technologies) is used and extracted DNA is added to the real-time PCR mixture containing 0.3 µM of each primer and 0.1 µM probe to a final volume of 10 µl. Real-time PCR conditions consist of 20 seconds at 95°C, followed by 45 cycles of 3 seconds at 95°C and 30 seconds at 60°C. At the completion of the TaqMan real-time PCR assay, the presence of PirA DNA is demonstrated by the presence of specific amplicons, identified by software-generated characteristic amplification curves. No-template controls must have no evidence of specific amplicons. The primers and probe and target gene for the *Vp*$_{AHPND}$- specific real-time PCR are listed in Table 4.4.1.1.~~

~~***Table 4.4.1.1. Primers and probe for the real-time PCR method for detection of pirA toxin gene***~~

~~Primer/probe name~~	~~Sequence (5'–3')~~	~~Target gene~~	~~Reference~~
~~VpPirA-F~~	~~TTG-GAC-TGT-CGA-ACC-AAA-CG~~	~~pirA~~	~~Han *et al*., 2015b~~
~~VpPirA-R~~	~~GCA-CCC-CAT-TGG-TAT-TGA-ATG~~		
~~VpPirA Probe~~	~~FAM-AGA-CAG-CAA-ACA-TAC-ACC-TAT-CAT-CCC-GGA-TAMRA~~		

4.4.2. Conventional PCR

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1 (AP1): Flegel & Lo, 2014; GenBank : KP324996; 700 bp			

pVA1	Fwd AP1F: 5CCT-TGG-GTG-TGC-TTA-GAG-GAT-G Rev AP1R: GCA-AAC-TAT-CGC-GCA-GAA-CAC-C	0.2 µM each	94°C/5 min; 25–30 cycles of 94°C/30 sec, 60°C/30 sec and 72°C/60 sec; final extension step at 72°C/10 min. Reaction mixture can be held at 4°C
Method 2 (AP2): Flegel & Lo, 2014; GenBank : KP324996; 700 bp			
pVA1	Fwd AP2F: TCA-CCC-GAA-TGC-TCG-CTT-GTG-G Rev AP2R: CGT-CGC-TAC-TGT-CTA-GCT-GAA-G	0.2 µM each	94°C/5 min; 25–30 cycles of 94°C/30 sec, 60°C/30 sec, 72°C/60 sec; final extension step at 72°C/10 min. Reaction mixture can be held at 4°C
Method 3 (AP3): Sirikharin *et al.*, 2015; GenBank: JALL01000066.1; 333 bp			
pirA^{vp}	Fwd AP3-F: ATG-AGT-AAC-AAT-ATA-AAA-CAT-GAA-AC Rev AP3-R: GTG-GTA-ATA-GAT-TGT-ACA-GAA	0.2 µM each	94°C/5 min; 30 cycles of 94°C/30 sec, 53°C/30 sec, 72°C/40 sec; final elongation step at 72°C/7 min; Reaction mixture can be held at 4°C
Method 4 (TUMSAT-Vp3): Tinwongger *et al.*, 2014; GenBank : AB972427; 360 bp			
pVA1	Fwd TUMSAT-Vp3 F: GTG-TTG-CAT-AAT-TTT-GTG-CA Rev TUMSAT-Vp3 R: TTG-TAC-AGA-AAC-CAC-GAC-TA	0.6 µM each	95°C/2 min; 30 cycles of 95°C/30 sec, 56°C/30 sec, 72°C/30 sec
Method 5 (VpPirA-284): Han *et al.*, 2015a; GenBank : KM067908; 284 bp			
pirA^{vp}	Fwd VpPirA-284F: TGA-CTA-TTC-TCA-CGA-TTG-GAC-TG Rev VpPirA-284R: CAC-GAC-TAG-CGC-CAT-TGT-TA	0.2 µM each	94°C/3 min; 35 cycles of 94°C/30 sec, 60°C/30 sec, 72°C/30 sec; final extension 72°C/7 min
Method 6 (VpPirB-392): Han *et al.*, 2015a; GenBank KM067908; 392 bp			
pirB^{vp}	Fwd VpPirB-392F: TGA-TGA-AGT-GAT-GGG-TGC-TC Rev VpPirB-392R: TGT-AAG-CGC-CGT-TTA-ACT-CA	0.2 µM each	94°C/3 min; 35 cycles of 94°C/30 sec, 60°C/30 sec, 72°C/30 sec; final extension 72°C/7 min
Method 7 (AP4): Dangtip *et al.*, 2015; GenBank : JPKS01000000; 1269 bp			
PirA and PirB toxin genes	Fwd AP4-F1: ATG-AGT-AAC-AAT-ATA-AAA-CAT-GAA-AC Rev AP4-R1: ACG-ATT-TCG-ACG-TTC-CCC-AA	0.2 µM each	94°C/2 min; 30 cycles of 94°C/30 sec, 55°C/30 sec, 72°C/90 sec; final extension step at 72°C/2 min; hold at 4°C
Method 8 (AP4): Dangtip *et al.*, 2015; GenBank : JPKS01000000; 230 bp			
PirA and PirB toxin genes	Fwd AP4-F2: TTG-AGA-ATA-CGG-GAC-GTG-GG Rev AP4-R2: GTT-AGT-CAT-GTG-AGC-ACC-TTC	0.2 µM each	94°C/2 min; 25 cycles of 94°C/20 sec, 55°C/20 sec, 72°C/20 sec; hold at 4°C

~~**One-step PCR detection of pVA1 plasmid**~~

~~Two one-step PCR methods (AP1 and AP2) are described here for detection of the pVA1 plasmid in enrichment broth cultures. The primers, target gene and the size of the expected amplicons are listed in Table 4.4.2.1.~~

Table 4.4.2.1. PCR primers for one-step PCR detection of pVA1 plasmid

Method name	Primers (5'–3')	Target gene	Expected amplicon size	Reference
AP1	AP1F: 5CCT TGG GTG TGC TTA GAG GAT G AP1R: GCA AAC TAT CGC GCA GAA CAC C	*pVA1*	700bp	Flegel & Lo (2014)
AP2	AP2F: TCA CCC GAA TGC TCG CTT GTG G AP2R: CGT CGC TAC TGT CTA GCT GAA G	*pVA1*	700bp	Flegel & Lo (2014)

Protocol for the AP1 and AP2 PCR methods

This protocol follows the method described by Flegel & Lo (2014). The PCR reaction mixture consists of 2.5 µl 10× PCR mix, 0.7 µl 50 mM $MgCl_2$, 0.4 µl 10 mM dNTPs, 0.5 µl 10 µM AP1/AP2F, 0.5 µl 10 µM AP1/AP2R, 0.2 µl Taq DNA polymerase and approximately 0.01–1 ng of template DNA in a total volume of 25 µl made up with distilled water. For PCR a denaturation step of 94°C for 5 minutes is followed by 25–30 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 60 seconds with a final extension step at 72°C for 10 minutes and then the reaction mixture can be held at 4°C (https://enaca.org/publications/health/disease-cards/ahpnd-detection-method-announcement.pdf).

One-step PCR detection of PirA/PirB toxin genes

Four one-step PCR methods (AP3, TUMSAT-Vp3, VpPirA-284 and VpPirB-392) are described here for detection of Pir toxin genes in enrichment broth cultures. The primers, target gene and the size of the expected amplicons are listed in Table 4.4.2.32.

Table 4.4.2.2. PCR primers for one-step PCR detection of PirA and PirB toxin genes

Method name	Primers (5'–3')	Target gene	Expected amplicon size	Reference
AP3	AP3-F: ATG AGT AAC AAT ATA AAA CAT GAA AC AP3-R: GTG GTA ATA GAT TGT ACA GAA	*pirA*^{vp}	333bp	Sirikharin *et al.*, 2015
TUMSAT-Vp3	TUMSAT-Vp3-F: GTG TTG CAT AAT TTT GTG CA TUMSAT-Vp3-R: TTG TAC AGA AAC CAC GAC TA	*pirA*^{vp}	360bp	Tinwongger *et al.*, 2014
VpPirA-284	VpPirA-284F: TGA CTA TTC TCA CGA TTG GAC TG VpPirA-284R: CAC GAC TAG CGC CAT TGT TA	*pirA*^{vp}	284bp	Han *et al.*, 2015a
VpPirB-392	VpPirB-392F: TGA TGA AGT GAT GGG TGC TC VpPirB-392R: TGT AAG CGC CGT TTA ACT CA	*pirB*^{vp}	392bp	Han *et al.*, 2015a

Protocol for the AP3 PCR method

This protocol follows the method described by Sirikharin *et al.* (2015). The PCR reaction mixture consists of 2.5 µl 10× PCR mix, 0.7 µl 50 mM $MgCl_2$, 0.4 µl 10 mM dNTPs, 0.5 µl 10 µM AP3-F1, 0.5 µl 10 µM AP3-R1, 0.2 µl Taq DNA polymerase and approximately 100 ng of template DNA in a total volume of 25 µl made up with distilled water. For PCR a denaturation step of 94°C for 5 minutes is followed by 30 cycles of 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 40 seconds with a final extension step at 72°C for 5 minutes and then the reaction mixture can be held at 4°C.

Protocol for the VpPirA-284 and VpPirB-392 PCR methods

This protocol follows the method described by Han *et al.* (2015a) and uses PuReTaq ready-to-go PCR beads (GE Healthcare). A 25 µl PCR reaction mixture is prepared with PuReTaq ready-to-go PCR beads. Each reaction contains 0.2 µM of each primer, 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U of Taq DNA polymerase, and 1 µl of extracted DNA. For PCR a 3-minute denaturation step at 94°C is followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 minutes.

Protocol for the TUMSAT-Vp3 PCR method

This protocol follows the method described by Tinwongger *et al.* (2014). A 30 µl PCR mixture is prepared containing 1 µl DNA template, 10× PCR buffer, 0.25 mM dNTP mixture, 0.6 µM of each primer and 0.01 U Taq

polymerase. PCR conditions consist of an initial preheating stage of 2 minutes at 95°C, followed by 30 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 56°C and 30 seconds extension at 72°C.

AP4 nested PCR protocol for detection of Vp_{AHPND}

This protocol follows the method described by Dangtip *et al.* (2015). The first PCR reaction mixture consists of 2.5 µl 10× PCR mix, 1.5 µl 50 mM $MgCl_2$, 0.5 µl 10 mM dNTPs, 0.5 µl 10 µM AP4-F1, 0.5 µl 10 µM AP4-R1, 0.3 µl of Taq DNA pol (5 units μl^{-1}) and approximately 100 ng of template DNA in a total volume of 25 µl made up with distilled water. The PCR protocol is 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 90 seconds with a final extension step at 72°C for 2 minutes and hold at 4°C.

The nested PCR reaction mixture consists of 2.5 µl 10x PCR mix, 1.5 µl 50 mM $MgCl_2$, 0.5 µl 10 mM dNTPs, 0.375 µl 10 µM AP4-F2, 0.375 µl 10 µM AP4-R2, 0.3 µl Taq DNA pol (5 units μl^{-1}) and 2 µl of the first PCR reaction in a total volume of 25 µl. The nested PCR protocol is 94°C for 2 minutes followed by 25 cycles of 94°C for 20 seconds, 55°C for 20 seconds and 72°C for 20 seconds and hold at 4°C.

The nested PCR primers, designed using the China (People's Rep. of) isolate of AHPND bacteria (Yang *et al.*, 2014), are shown in Table 4.4.2.73. The expected amplicon sizes are 1269 bp for the outer primers (AP4-F1 and AP4-R1) and 230 bp for the inner primers (AP4-F2 and AP4-R2). At high concentrations of target DNA, additional amplicons may occur as the product of residual primer AP4-F1 pairing with AP4-R2 (357 bp) or AP4-F2 with AP4-R1 (1142 bp) in the nested step.

Table 4.4.2.3. Primers for the AP4, nested PCR method for detection of PirA and PirB toxin genes

Method name	Primers (5'-3')	Expected amplicon size	Reference
AP4 Step 1	AP4-F1: ATG-AGT-AAC-AAT-ATA-AAA-CAT-GAA-AC AP4-R1: ACG-ATT-TCG-ACG-TTC-CCC-AA	1269	Dangtip *et al.*, 2015
AP4 Step 2	AP4-F2: TTG-AGA-ATA-CGG-GAC-GTG-GG AP4-R2: GTT-AGT-CAT-GTG-AGC-ACC-TTC	230	

Analysis of conventional PCR products by agarose gel electrophoresis

After PCR, amplicons are visualised by agarose gel electrophoresis. Twenty µl of the PCR reaction mixture, with 6× loading dye added, is loaded onto a 1.5% agarose gel and electrophoresis is carried out at 90 volts for 40 minutes. Amplicons are visualised with SYBR Safe gel stain (Invitrogen, Cat. No. 33102) according to the manufacturer's instructions. Amplicons of the expected size appropriate for the PCR methods used (Tables 4.4.2.1, 4.4.2.2 and 4.4.2.3) indicate a positive result.

4.4.3. Other nucleic acid amplification methods

Cruz-Flores *et al.* (2019) developed a multiplex real-time PCR-based SYBR green assay for simultaneous detection of *pirA*, *pirB*, 16S rRNA and 18S rRNA, and a duplex real-time PCR-based Taqman probe assay showing high specificity and sensitivity – limit of detection was 10 copies for both *pirA and pirB*.

A recombinase polymerase amplification assay was developed by Mai *et al.* (2021). This assay has a limit of detection of five copies of the *pirAB* gene and high specificity. A LAMP-based assay for AHPND detection developed by Koiwai *et al.* (2016) also shows high specificity and sensitivity.

4.5. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced, analysed and compared with published sequences.

The positive results obtained from conventional PCR described in 4.4.2 need to be confirmed by sequencing.

4.6. *In-situ* hybridisation

ISH is Not currently available (December 2021).

4.7. Immunohistochemistry

An immunohistochemistry assay to detect AHPND was developed by Kumar *et al.,* (2019). However, the assay requires further validation.

4.8. Bioassay

Vp$_{AHPND}$ has been transmitted experimentally by immersion and by reverse gavage (Joshi *et al*., 2014b; Nunan *et al.,* 2014; Soto-Rodriguez *et al.,* 2015; Tran *et al.,* 2013), simulating natural horizontal transmission via oral routes and co-habitation. Thus, following isolation and purification of a bacterium that is suspected to cause AHPND, a bioassay can be performed to confirm the presence of the causative agent. The immersion procedure is carried out by immersing 15 shrimp for 15 minutes, with aeration, in a suspension (150 ml clean artificial seawater) of 2×10^8 cells of the cultured bacterium per ml. Following this initial 15-minute period, the shrimp and the inoculum are transferred to a larger tank with a volume of clean artificial seawater to make the final concentration of the bacterium 2×10^6 cells ml^{-1}. Shrimp are monitored at 6- to 8-hour intervals. Dead shrimp can be processed for *Vp*$_{AHPND}$ PCR and sequence analysis. Moribund or surviving shrimp are processed for histology, bacterial re-isolation, PCR and sequence analysis. A positive bioassay is indicated by the detection of characteristic histological lesions and *Vp*$_{AHPND}$ by PCR and amplicon sequence analysis.

4.9. Antibody- or antigen-based detection methods

An indirect enzyme-linked immunosorbent assay (I-ELISA) for AHPND detection developed by Mai *et al.* (2020) showed high sensitivity (the limit of detection was 0.008 ng μl^{-1} for PirA^{vp} and 0.008 ng μl^{-1} for PirB^{vp}) and specificity.

4.10. Other methods

None.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR (Han *et al.,* 2015b) and conventional PCR (Dangtip *et al*., 2015) are ~~is~~ recommended for demonstrating freedom from AHPND in an apparently healthy population.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for suspect and confirmed cases have been developed to support decision-making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [4]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. ~~Geographical~~ Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with AHPND shall be suspected if at least one of the following criteria is met:

[4] For example transboundary commodities.

i) A positive result by ~~any of the~~ real-time PCR

ii) A positive result by ~~or~~ conventional PCR ~~methods recommended in Table 4.1~~

iii) A positive result by LAMP

iv) Histopathology ~~or cytopathological changes~~ consistent with the ~~presence of the pathogen or the~~ disease

v) A positive result by Ag-ELISA

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *Vibrio parahaemolyticus* (*Vp*$_{AHPND}$) is considered to be confirmed if at least one of the following ~~criterion~~ criteria is met:

i) Positive results by real-time PCR and conventional PCR followed by amplicon sequence analysis

ii) Positive results by LAMP and conventional PCR followed by amplicon sequence analysis

iii) Positive results by Ag-ELISA and conventional PCR followed by amplicon sequence analysis

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *Vibrio parahaemolyticus* (*Vp*$_{AHPND}$) shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) A positive result by real-time PCR

iii) A positive result by conventional PCR

iv) A positive result by LAMP

v) A positive result by Ag-ELISA

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *Vibrio parahaemolyticus* (*Vp*$_{AHPND}$) is considered to be confirmed if at least one of the following ~~criterion~~ criteria is met:

i) Positive results by real-time PCR and conventional PCR followed by amplicon sequence analysis.

ii) Positive results by LAMP and conventional PCR followed by amplicon sequence analysis

iii) Positive results by Ag-ELISA and conventional PCR followed by amplicon sequence analysis

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *Vibrio parahaemolyticus* (*Vp*$_{AHPND}$) are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with *Vibrio parahaemolyticus* (*Vp*$_{AHPND}$), however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Conventional PCR	Diagnosis	Clinically diseased and apparently healthy shrimp	AHPND causing and non-causing bacterial isolates	*Penaeus vannamei*	100	100	Bioassay	Sirikharin *et al.*, 2015
Conventional PCR	Diagnosis	Clinically diseased and apparently healthy shrimp	AHPND causing and non-causing bacterial isolates	NA	100[1]	100	Bioassay	Tinwongger *et al.*, 2014
Real-time PCR	Diagnosis	Clinically diseased animals	Hepato-pancreas	*Penaeus vannamei*	100	NA	Bioassay and histopathology	Han *et al.* 2015b

DSe = diagnostic sensitivity, DSp = diagnostic specificity, NA= Not available, PCR: = polymerase chain reaction.
[1]100% sensitivity for TUMSAT-Vp3 primer set.

6.3.2. For surveillance of apparently healthy animals

Test type	Test rpose	Source pulations	Tissue or mple types	Species	DSe)	DSp)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, NA= Not available, PCR: = polymerase chain reaction.

7. References

ARANGUREN CARO L.F, MAI H.N., KANRAR S., CRUZ-FLORES R. & DHAR A.K. (2020a). A mutant of *Vibrio parahaemolyticus pir*AB$_{VP}$ (+) that carries binary toxin genes but does not cause acute hepatopancreatic necrosis disease. *Microorganisms*, **8**, 1549.

Aranguren Caro L.F., Mai H.N., Noble B. & Dhar A.K. (2020b). Acute hepatopancreatic necrosis disease (VP_{AHPND}), a chronic disease in shrimp (*Penaeus vannamei*) population raised in latin America. J. Invertebr. Pathol., 174, 107424. doi: 10.1016/j.jip.2020.107424. Epub 2020 Jun 11.PMID: 32535000

CRUZ-FLORES R., MAI H.N & DHAR A.K. (2019). Multiplex SYBR Green and duplex TaqMan real-time PCR assays for the detection of *Photorhabdus* Insect-Related (Pir) toxin genes *pirA* and *pirB*. *Mol. Cell. Probes*, **43**, 20–28.

DABU I.M., LIM J.J., ARABIT P.M.T., ORENSE S.J.A.B., TABARDILLO J.A., CORRE V.L. & MANINGAS M.B.B. (2017). The first record of acute hepatopancreatic necrosis disease in the Philippines. *Aquacult. Res.*, **48**, 792–799.

DANGTIP S., SIRIKHARIN R, SANGUANRUT P., THITAMADEE S, SRITUNYALUCKSANA K., TAENGCHAIYAPHUM S., MAVICHAK R., PROESPRAIWONG P. & FLEGEL T.W. (2015). AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*. *Aquaculture Rep.*, **2**, 158–162.

DE LA PENA L.D., CABILLON N.A.R., CATEDRAL D.D., AMAR E.C., USERO R.C., MONOTILLA W.D., CALPE A.T., FERNANDEZ D.D. & SALOMA C.P. (2015). Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *P. monodon* cultured in the Philippines. *Dis. Aquat. Org.*, **116**, 251–254.

FLEGEL T.W. & LO C.F. (2014). Free release of primers for specific detection of bacterial isolates that cause acute hepatopancreatic necrosis disease (AHPND). Published by the Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand. https://enaca.org/enclosure/?id=88

GOMEZ-GIL B., SOTO-RODRÍGUEZ S., LOZANO R. & BETANCOURT-LOZANO M. (2014). Draft genome sequence of *Vibrio parahaemolyticus* strain M0605, which causes severe mortalities of shrimps in Mexico. *Genome Announc.*, **2**, e00055-14.

GOMEZ-JIMENEZ S., NORIEGA-OROZCO L., SOTELO-MUNDO R.R., CANTU-ROBLES V.A., COBIAN-GUEMES A.G., COTA-VERDUGO R.G., GAMEZ-ALEJO L.A., DEL POZO-YAUNER L., GUEVARA-HERNANDEZ E., GARCIA-OROZCO K.D., LOPEZ-ZAVALA A.A. & OCHOA-LEYVA A. (2014). High-quality draft genomes of two *Vibrio parahaemolyticus* strains aid in understanding acute hepatopancreatic necrosis disease of cultured shrimps in Mexico. *Genome Announc.*, **2**, e00800-14.

HAN J.E., TANG K.F.J., TRAN L.H. & LIGHTNER D.V. (2015a). *Photorhabdus* insect related (*Pir*) toxin-like genes in a plasmid of *Vibrio parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis disease (AHPND) of shrimp. *Dis. Aquat. Org.*, **113**, 33–40.

HAN J.E., TANG K.F.J., PANTOJA C.R., WHITE B.L. & LIGHTNER D.V. (2015b). qPCR assay for detecting and quantifying a virulence plasmid in acute hepatopancreatic necrosis disease (AHPND) due to pathogenic *Vibrio parahaemolyticus*. *Aquaculture*, **442**, 12–15.

HONG X.P., XU D., ZHUO Y., LIU H.Q. & LU L.Q. (2016). Identification and pathogenicity of *Vibrio parahaemolyticus* isolates and immune responses of *Penaeus* (*Litopenaues*) *vannamei* (Boone). *J. Fish Dis.,* **39**, 1085–1097.

JOSHI J., SRISALA J., SAKAEW W., PRACHUMWAT A., SRITUNYALUCKSANA K., FLEGEL T.W. & THITAMADEE S. (2014a). Identification of bacterial agent(s) for acute hepatopancreatic necrosis syndrome, a new emerging shrimp disease. *Suranaree J. Sci. Technol*. Available from: http://ird.sut.ac.th/e-journal/ Journal/pdf/140283.pdf.

JOSHI J., SRISALA J., TRUONG V.H., CHEN I.T., NUANGSAENG B., SUTHIENKUL O., LO C.F., FLEGEL T.W., SRITUNYALUCKSANA K. & THITAMADEE S. (2014b). Variation in *Vibrio parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND). *Aquaculture*, **428–429**, 297–302.

KARUNASAGAR I., KARUNASAGAR I., VENUGOPAL M.N. & NAGESHA C.N. (1987). Survival of *Vibrio parahaemolyticus* in estuarine and sea water and in association with clams. *Syst. Appl. Microbiol*., **9**, 316–319.

KOIWAI K., TINWONGGER S., NOZAKI R., KONDO H. & HIRONO I. (2016). Detection of acute hepatopancreatic necrosis disease strain of *Vibrio parahaemolyticus* using loop-mediated isothermal amplification. *J. Fish Dis*., **39**, 603–606.

KONDO H., TINWONGGER S., PROESPRAIWONG P., MAVICHAK R., UNAJAK S., NOZAKI R. & HIRONO I. (2014). Draft genome sequences of six strains of *Vibrio parahaemolyticus* isolated from early mortality syndrome/acute hepatopancreatic necrosis disease shrimp in Thailand. *Genome Announc.*, **2**, e00221-14.

KONDO H., VAN P.T., DANG L.T. & HIRONO I. (2015). Draft genome sequences of non-*Vibrio parahaemolyticus* acute hepatopancreatic necrosis disease strain KC13.17.5, isolated from diseased shrimp in Vietnam. *Genome Announc.*, **3**, e00978-15.

KUMAR V., BELS L.D., COUCK L., BARUAH K., BOSSIER P. & BROECK W.V.D. (2019). PirABVP Toxin Binds to Epithelial Cells of the Digestive Tract and Produce Pathognomonic AHPND Lesions in Germ-Free Brine Shrimp. *Toxins*, **11**, 717.

LEE C.T., CHEN I.T., YANG Y.T., KO T.P., HUANG Y.T., HUANG J.Y., HUANG M.F., LIN S.J., CHEN C.Y., LIN S.S., LIGHTNER D.V., WANG A.H., WANG H.C., HOR L.I. & LO C.F. (2015). The opportunistic marine pathogen *Vibrio parahaemolyticus* becomes virulent by acquiring a plasmid that expresses a deadly toxin. *Proc. Natl Acad. Sci. USA*., **112**, 10798–10803.

LIGHTNER D.V. (1996). A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquaculture Society, Baton Rouge, LA, USA.

LO C.-F., LEU J.-H., HO C.-H., CHEN C.-H., PENG S.-E., CHEN Y.-T., CHOU C.-M., YEH P.-Y., HUANG C.-J., CHOU H.-Y., WANG C.-H. & KOU G.-H. (1996). Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Dis. Aquat. Org.,* **25**, 133–141.

MAI N.H., ARANGUREN L.F.C, CRUZ-FLORES R. & DHAR A.K. (2021). Development of a Recombinase Polymerase Amplification (RPA) assay for acute hepatopancreatic necrosis disease (AHPND) detection in Pacific white shrimp (*Penaeus vannamei*). *Mol. Cell. Probes*, **57**, 101710.

MAI H.N., CRUZ-FLORES R. & DHAR A.K. (2020). Development of an indirect Enzyme Linked Immunoassay (iELISA) using monoclonal antibodies against Photorhabdus insect related toxins, PirA^{Vp} and PirB^{Vp} released from *Vibrio* spp. *J. Microbiol. Methods*, **176**, 106002.

MUNTADA-GARRIGA J.M., RODRIGUEZ-JEREZ J.J., LOPEZ-SABATER E.I. & MORA-VENTURA M.T. (1995). Effect of chill and freezing temperatures on survival of *Vibrio parahaemolyticus* inoculated in homogenates of oyster meat. *Lett. Appl. Microbiol*., **20**, 225–227.

NACA (2014). Acute hepatopancreatic necrosis disease card (updated June 2014). Published by the Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

NUNAN L., LIGHTNER D., PANTOJA C. & GOMEZ-JIMENEZ S. (2014). Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. *Dis. Aquat. Org.,* **111**, 81–86.

~~POWERS Q.M., ARANGUREN L.F., FITZSIMMONS K.M., MCLAIN J.E. & DHAR A.K. (2021). Crayfish (*Cherax quadricarinatus*) susceptibility to acute hepatopancreatic necrosis disease (AHPND). *J. Invertebr. Pathol*., **186**, 107554.~~

~~SCHOFIELD P.J., NOBLE B.L, ARANGUREN CARO L.F., MAI H.N., PADILLA T.J, MILLABAS J. & DHAR A.K. (2020). Pathogenicity of Acute Hepatopancreatic Necrosis Disease (AHPND) on the freshwater prawn, *Macrobrachium rosenbergii*, and Pacific White Shrimp, *Penaeus vannamei*, at various salinities. *Aquac. Res*., **52**, 1480–1489.~~

SIRIKHARIN R., TAENGCHAIYAPHUM S., SANGUANRUT P., CHI T.D., MAVICHAK R., PROESPRAIWONG P., NUANGSAENG B., THITAMADEE S., FLEGEL T.W. & SRITUNYALUCKSANA K. (2015). Characterization and PCR detection of binary, Pir-like toxins from *Vibrio parahaemolyticus* isolates that cause acute hepatopancreatic necrosis disease (AHPND) in shrimp. *PLoS ONE*, **10**, e0126987. doi:10.1371/journal.pone.0126987.

SOTO-RODRIGUEZ S.A., GOMEZ-GIL B., LOZANO-OLVERA R., BETANCOURT-LOZANO M. & MORALES-COVARRUBIAS M.S. (2015). Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*) in northwestern Mexico. *Appl. Environ. Microbiol*., **81**, 1689–1699.

THOMSON W.K. & THACKER C.L. (1973). Effect of temperature on *Vibrio parahaemolyticus* in oysters at refrigerator and deep freeze temperatures. *Can. Inst. Food Sci. Tech. J*., **6**, 156–158.

TINWONGGER S., PROESPRAIWONG P., THAWONSUWAN J., SRIWANAYOS P., KONGKUMNERD J., CHAWEEPACK T., MAVICHAK R., UNAJAK S., NOZAKI R., KONDO H. & HIRONO I. (2014). Development of PCR diagnosis method for shrimp acute hepatopancreatic necrosis disease (AHPND) strain of *Vibrio parahaemolyticus*. *Fish Pathol.,* **49**, 159–164.

TRAN L.H., FITZSIMMONS K. & LIGHTNER D.V. (2014). AHPND/EMS: From the academic science perspective to the production point of view. *Aquaculture Asia Pacific*, **10**, 14–18.

TRAN L., NUNAN L., REDMAN R.M., MOHNEY L.L., PANTOJA C.R., FITZSIMMONS K. & LIGHTNER D.V. (2013). Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Dis. Aquat. Org*., **105**, 45–55.

WEISBURG W.G., BARNS S.M., PELLETIER D.A. & LANE D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*., **173**, 697–703.

YANG Y.T., CHEN I.T., LEE C.T., CHEN C.Y., LIN S.S., HOR L.I., TSENG T.C., HUANG Y.T., SRITUNYALUCKSANA K., THITAMADEE S., WANG H.C. & LO C.F. (2014). Draft genome sequences of four strains of *Vibrio parahaemolyticus*, three of which cause early mortality syndrome/acute hepatopancreatic necrosis disease in shrimp in China and Thailand. *Genome Announc*., **2**, e00816-14.

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NB: There are OIE Reference Laboratories for acute hepatopancreatic necrosis disease (please consult the OIE web site for the most up-to-date list: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

Please contact the OIE Reference Laboratory for any further information on acute hepatopancreatic necrosis disease

NB: FIRST ADOPTED IN 2017; MOST RECENT UPDATES ADOPTED IN 2018.

CHAPTER 2.2.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

1. Scope

Infection with *Aphanomyces astaci* means infection with the pathogenic agent *A. astaci*, Phylum Oomycota. The disease is commonly known as crayfish plague.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Aphanomyces astaci is a water mould. The Oomycetida or Oomycota, are considered protists and are classified with diatoms and brown algae in a group called the Stramenopiles or Chromista.

Five groups (A–E) of *A. astaci* have been described based on random amplification of polymorphic DNA polymerase chain reaction (RAPD PCR) (Dieguez-Uribeondo *et al.,* 1995; Huang *et al.,* 1994; Kozubikova *et al.,* 2011). Additional geno- or haplotypes are still being detected using molecular methods (Di Domenico *et al*., 2021). Group A (the so called *Astacus* strains) comprises strains isolated from several European crayfish species. These strains are thought to have been in Europe for a long period of time. Group B (*Pacifastacus* strains I) includes isolates from several European crayfish species and from the invasive *Pacifastacus leniusculus* in Europe as well as Lake Tahoe, USA. Imported to Europe, *P. leniusculus* probably introduced this genotype of *A. astaci* and infected the native European crayfish. Group C (*Pacifastacus* strains II) consists of a strain isolated from *P. leniusculus* from Pitt Lake, Canada. Another strain (Pc), isolated from *Procambarus clarkii* in Spain, sits in group D (*Procambarus* strains). This strain shows temperature/growth curves with higher optimum temperatures compared with groups A and B (Dieguez-Uribeondo *et al*., 1995). *Aphanomyces astaci* strains that have been present in Europe for many years (group A strains) appear to be less pathogenic than strains introduced with crayfish imports from North America since the 1960s.

2.1.2. Survival and stability in processed or stored samples

Aphanomyces astaci is poorly resistant against desiccation and does not survive long in decomposing hosts. Any treatment of the crayfish (freezing, cooking, drying) will affect the survival of the pathogen (Oidtmann *et al.,* 2002). Isolation from processed samples is not possible, however they may be suitable for molecular methods used for pathogen detection.

2.1.3. Survival and stability outside the host

Outside the host *Aphanomyces astaci* is found as zoospores that remain motile for up to 3 days and form cysts that can survive for 2 weeks in distilled water. As *A. astaci* can go through three cycles of encystment and zoospore emergence, the maximum life span outside of a host could be several weeks. Spores remained viable in a spore suspension in clean water kept at 2°C for 2 months (Unestam, 1966). Survival time is probably shorter in natural waters.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

All stages of crayfish species native to Europe, including the noble crayfish (*Astacus astacus*) of north-west Europe, the white clawed crayfish (*Austropotamobius pallipes*) of south-west and west Europe, the related *Austropotamobius torrentium* (mountain streams of south-west Europe) and the slender clawed or Turkish crayfish (*Pontastacus leptodactylus*) of eastern Europe and Asia Minor are highly susceptible (e.g. Holdich *et al*., 2009). Australian species of crayfish are also highly susceptible. North American crayfish such as the signal crayfish (*Pacifastacus leniusculus*), Louisiana swamp crayfish (*Procambarus clarkii*) and *Faxonius* spp. are infected by *A. astaci*, but under normal conditions the infection does not cause clinical disease or death. All North American crayfish species investigated to date have been shown to be susceptible to infection, demonstrated by the presence of the pathogen in host cuticle (reviewed by Svoboda *et al*. 2017) and it is therefore currently assumed that this is the case for any other North American species.

The only other crustacean known to be susceptible to infection by *A. astaci* is the Chinese mitten crab (*Eriocheir sinensis*).

2.2.2. Species with incomplete evidence for susceptibility

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

The host species susceptible to infection with *A. astaci* fall largely into two categories: those highly susceptible to infection with development of clinical disease and mortalities, and those that are infected without associated clinical disease or mortalities.

Highly susceptible species: clinical disease outbreaks caused by infection with *A. astaci* are generally known as 'crayfish plague' outbreaks. In such outbreaks, moribund and dead crayfish of a range of sizes (and therefore ages) can be found.

2.2.4. Distribution of the pathogen in the host

The tissue that becomes initially infected is the exoskeleton cuticle. Soft cuticle, as is found on the ventral abdomen and around joints, is preferentially affected. In the highly susceptible European crayfish species, the pathogen often manages to penetrate the basal lamina located underneath the epidermis cell layer. From there, *A. astaci* spreads throughout the body primarily by invading connective tissue and haemal sinuses; however, all tissues may be affected.

In North American crayfish species, infection is usually restricted to the cuticle. Based on PCR results, the tailfan (consisting of uropods and telson) and soft abdominal cuticle appear to be frequently infected (Oidtmann *et al.,* 2006; Vralstad *et al.,* 2011).

2.2.5. Aquatic animal reservoirs of infection

North American crayfish species act mostly as carriers of the infection without showing clinical signs. However, some strains, especially from group A, show lowered virulence, thus enabling normally highly susceptible European crayfish to act as carriers as well (see review by Svoboda *et al*., 2017).

Colonisation of habitats, initially occupied by highly susceptible species, by North American crayfish species carrying *A. astaci* is likely to result in an epizootic among the highly susceptible animals.

2.2.6. Vectors

Transportation of finfish may facilitate the spread of *A. astaci* through the presence of spores in the transport water or co-transport of infected crayfish specimens (Alderman *et al*., 1987; Oidtmann *et al*., 2002). There is also circumstantial evidence of spread by contaminated equipment (e.g. nets, boots, clothing, traps) (Alderman *et al*., 1987).

2.3. Disease pattern

2.3.1. Mortality, morbidity, and prevalence

When the infection first reaches a naïve population of highly susceptible crayfish species, high levels of mortality are usually observed within a short space of time, so that in areas with high crayfish densities the bottoms of

lakes, rivers and streams are covered with dead and dying crayfish. A band of mortality will spread quickly from the initial outbreak site downstream, whereas upstream spread is slower. Lower water temperatures are associated with slower mortalities and a greater range of clinical signs in affected animals (Alderman *et al*., 1987). Observations from Finland suggest that at low water temperatures, noble crayfish (*Astacus astacus*) can be infected for several months without the development of noticeable mortalities (Viljamaa-Dirks *et al*., 2013).

On rare occasions, single specimens of the highly susceptible species have been found after a wave of infection with *A. astaci* has gone through a river or lake. This is most likely to be due to lack of exposure of these animals during an outbreak (animals may have been present in a tributary of a river or lake or in a part of the affected river/lake that was not reached by spores, or crayfish may have stayed in burrows during the epizootic). However, low virulent strains of *A. astaci* have been described to persist in a water way, kept alive by a weak infection in the remnant population (Viljamaa-Dirks *et al*., 2011). Although remnant populations of susceptible crayfish species remain in many European watersheds, the dense populations that existed 150 years ago are now heavily diminished (Souty-Grosset *et al*., 2006; Holdich *et al*., 2009). Populations of susceptible crayfish may re-establish, but once population density and geographical distribution is sufficient for susceptible animals to come into contact with spores, new outbreaks of infection with *A. astaci* and large-scale mortalities will occur.

In the highly susceptible European crayfish species, exposure to *A. astaci* spores usually leads to infection and eventually to death. Prevalence of infection within a population in the early stage of an outbreak may be low (few animals in a river population may be affected). However, the pathogen is amplified in affected animals and subsequently released into the water; usually leading to 100% mortality in a contiguous population. The rate of spread from initially affected animals depends on several factors, one being water temperature. Therefore, the time from first introduction of the pathogen into a population to noticeable crayfish mortalities can vary greatly and may range from a few weeks to months. Prevalence of infection will gradually increase over this time and usually reach 100%. Data from a noble crayfish population in Finland that experienced an acute mortality event due to infection with *A. astaci* in 2001 suggest that in sparse noble crayfish populations, spread of disease throughout the host population may take several years (Viljamaa-Dirks *et al.,* 2011).

2.3.2. Clinical signs, including behavioural changes

Susceptible species

Gross clinical signs are variable and depend on challenge severity and water temperatures. The first sign of an epizootic may be the appearance of crayfish during daylight (crayfish are normally nocturnal), some of which may show loss of co-ordination, falling onto their backs and remaining unable to right themselves. Occasionally, the infected animals can be seen trying to scratch or pinch themselves.

Often, however, the first sign of an outbreak may be the presence of large numbers of dead crayfish in a river or lake (Alderman *et al.,* 1987).

Infection with *A. astaci* may cause mass mortality of crayfish. However, investigation of mortality event should consider other causes such as environmental pollution (e.g. insecticides such as cypermethrin have been associated with initial misdiagnoses).

North American crayfish species

Infected North American crayfish may be subclinical carriers. Controlled exposure to a highly virulent strain has resulted in mortality in juvenile stages of *Pacifastacus leniusculus* as well as behavioural alterations in adults (Thomas *et al.,* 2020).

2.3.3 Gross pathology

Susceptible species

Depending on a range of factors, the foci of infection in crayfish may be seen by the naked eye or may not be discernible despite careful examination. Infection foci are best viewed under a low power stereo microscope and are recognisable by localised whitening of the muscle beneath the cuticle. In some cases, a brown colouration of cuticle and muscle may occur, or hyphae may be visible in infected cuticles in the form of fine brown (melanised) tracks in the cuticle. Sites for examination include the intersternal soft ventral cuticle of the abdomen and tail, the cuticle of the perianal region, the cuticle between the carapace and abdomen, the joints of the pereiopods (walking legs), particularly the proximal joint, eyestalks and finally the gills.

North American crayfish species

Infected North American crayfish can sometimes show melanised spots in their soft cuticle, for example, the soft abdominal cuticle and joints. These melanisations can be caused by mechanical injuries or infections with other water moulds and are non-specific. However, populations with high levels of infection can show abnormally high levels of cuticular damage in individual animals, such as missing legs and claws due to deteriorated joints.

2.3.4. Modes of transmission and life cycle

The main routes of spread of the pathogen are through 1) movement of infected crayfish, 2) movement of spores with contaminated water or equipment, as may occur during movements of finfish, or 3) through colonisation of habitats by invasive North American crayfish species.

The main route of spread of *A. astaci* in Europe between the 1960s and 2000 was through the active stocking of North American crayfish into the wild or escapes from crayfish farms. Subsequent spread occurs through expanding populations of invasive North American crayfish, accidental co-transport of specimens, and release of North American crayfish into the wild by private individuals (Holdich *et al.,* 2009).

Transmission from crayfish to crayfish occurs through the release of zoospores from an infected animal and attachment of the zoospores to naïve crayfish. The life cycle of *A. astaci* is simple with vegetative hyphae invading and ramifying through host tissues, eventually producing extramatrical sporangia that release amoeboid primary spores. These initially encyst, but then release a biflagellate zoospore (secondary zoospore). Biflagellate zoospores swim in the water column and, on encountering a susceptible host, attach and germinate to produce invasive vegetative hyphae. The zoospores of *A. astaci* swim actively in the water column and have been demonstrated to show positive chemotaxis towards crayfish (Cerenius & Söderhäll, 1984). Zoospores are capable of repeated encystment and re-emergence, extending the period of their infectivity (Soderhall & Cerenius 1999). Growth and sporulation capacity is strain-and temperature-dependent (Dieguez-Uribeondo *et al*., 1995).

2.3.5. Environmental factors

Under laboratory conditions, the preferred temperature range at which the *A. astaci* mycelium grows varies slightly depending on the strain. In a study, which compared several *A. astaci* strains that had been isolated from a variety of crayfish species, mycelial growth was observed between 4 and 29.5°C, with the strain isolated from *Procambarus clarkii* growing better at higher temperatures compared to the other strains. Sporulation efficiency was similarly high for all strains tested between 4 and 20°C, but it was clearly reduced for the non-*P. clarkii* strains at 25°C and absent at 27°C. In contrast, sporulation still occurred in the *P. clarkii* strain at 27°C. The proportion of motile zoospores (out of all zoospores observed in a zoospore suspension) was almost 100% at temperatures ranging from 4–18°C, reduced to about 60% at 20°C and about 20% at 25°C in all but the *P. clarkii* strain. In the *P. clarkii* strain, 80% of the zoospores were still motile at 25°C, but no motile spores were found at 27°C (Dieguez-Uribeondo *et al*., 1995).

Field observations show that outbreaks of infection with *A. astaci* occur over a wide temperature range, and at least in the temperature range 4–20°C. The rate of spread within a population depends on several factors, including water temperature. In a temperature range between 4 and 16°C, the speed of an epizootic is enhanced by higher water temperatures.

In buffered, redistilled water, sporulation occurs between pH 5 and 8, with the optimal range being pH 5–7. The optimal pH range for swimming of zoospores appears to be pH 6.0–7.5, with a maximum range between pH 4.5 and 9.0 (Unestam, 1966).

Zoospore emergence is influenced by the presence of certain salts in the water. $CaCl_2$ stimulates zoospore emergence from primary cysts, whereas $MgCl_2$ has an inhibitory effect. In general, zoospore emergence is triggered by transferring the vegetative mycelium into a medium where nutrients are absent or low in concentration (Cerenius *et al*., 1988).

2.3.6. Geographical distribution

In Europe the reports of large mortalities of crayfish go back to 1860. The reservoir of the original infections in the 19th century was never established. *Faxonius* (*Orconectes*) spp. were not known to have been introduced

into Europe until the 1890s, but the post-1960s extensions are largely linked to more recent introductions of North American crayfish for farming (Alderman, 1996; Holdich *et al*. 2009). *Pacifastacus leniusculus* and *Procambarus clarkii* are now widely naturalised in many parts of Europe.

In recent years, crayfish plague has been reported in Asia and also in North- and South America (see e.g. references in Di Domenico *et al.* 2021). The distribution of *A. astaci* in North America is likely to be much wider than reported.

Any geographical area where North American crayfish species were introduced must be considered as potentially infected if not proven otherwise. Lack of clinical disease in these carrier species may hamper the reliability in reporting the infection. For the highly susceptible species, see OIE WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level. However, even high mortalities can go unnoticed in wild populations.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No vaccines are available.

2.4.2. Chemotherapy including blocking agents

No treatments are currently known that can successfully treat the highly susceptible crayfish species, once infected.

2.4.3. Immunostimulation

No immunostimulants are currently known that can successfully protect the highly susceptible crayfish species against infection and consequent disease due to *A. astaci* infection.

2.4.4. Breeding resistant strains

A few studies suggest that there might be differences in resistance between populations of highly susceptible species (reviewed by Martin-Torrijos *et al*., 2017; Svoboda *et al*., 2017). The fact that North American crayfish generally do not develop clinical disease suggests that selection for resistance may be possible and laboratory studies using attenuated strains of *A. astaci* might be successful. However, there are currently no published data from such studies.

2.4.5. Inactivation methods

Aphanomyces astaci, both in culture and in infected crayfish, is inactivated by a short exposure to temperatures of 60°C or to temperatures of –20°C (or below) for 48 hours (or more) (Oidtmann *et al*., 2002). Sodium hypochlorite at 100 ppm, free chlorine and iodophors at 100 ppm available iodine, are effective for disinfection of contaminated equipment. Equipment must be cleaned prior to disinfection since organic matter decreases the effectiveness of disinfectants (Alderman & Polglase, 1985). Thorough drying of equipment (>24 hours) is also effective as *A. astaci* is not resistant to desiccation (Rennerfelt, 1936).

2.4.6. Disinfection of eggs and larvae

No information available.

2.4.7. General husbandry

If a crayfish farm for highly susceptible species is being planned, it should be carefully investigated whether North American crayfish species are in the vicinity of the planned site or present upstream . If North American crayfish are present, there is a high likelihood that susceptible farmed crayfish will eventually become infected.

In an endemic area where the highly susceptible species are being farmed, the following biosecurity recommendations should be followed to avoid an introduction of *A. astaci* onto the site:

1. General biosecurity should be in place (e.g. controlled access to premises; disinfection of boots when entering the site; investigation of mortalities if they occur; introduction of live animals (crayfish, finfish) only from sources known to be free from infection with *A. astaci*).

2. Movements of potentially infected live or dead crayfish, potentially contaminated water, equipment or any other item that might carry the pathogen from an infected to an uninfected site holding susceptible species should be prevented.

3. If transfers of finfish or crayfish are being planned, these should not come from streams or other waters that harbour potentially infected crayfish (either susceptible crayfish populations that are going through a current outbreak of infection with *A. astaci* or North American carrier crayfish).

4. North American crayfish should not be brought onto the site.

5. Finfish obtained from unknown freshwater sources or from sources, where North American crayfish may be present or a current outbreak of infection with *A. astaci* may be taking place, must not be used as bait or feed for crayfish, unless they have been subject to a temperature treatment to kill *A. astaci* (see Section 2.4.5. *Inactivation methods*).

6. Any equipment that is brought onto site should be disinfected.

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

For a suspected outbreak of infection with *A. astaci* in a population of highly susceptible crayfish species, sample crayfish should ideally consist of: a) live crayfish showing signs of disease, and b) live, apparently healthy crayfish. Freshly dead crayfish may also be suitable, although this will depend on their condition.

Live crayfish should be transported using insulated containers equipped with small holes to allow aeration. The temperature in the container should not exceed 16°C.

Crayfish should be transported in a moist atmosphere, for example using moistened wood shavings/wood wool, newspaper, or grass/hay. Unless transport water is sufficiently oxygenated, live crayfish should not be transported in water, as they may suffocate.

The time between sampling of live animals and delivery to the investigating laboratory should not exceed 24 hours.

Should only dead animals be found at the site of a suspected outbreak, freshly dead animals should be selected for diagnosis. Dead animals can either be: a) transported chilled (if they appear to have died only very recently), or, b) placed in non-methylated ethanol (minimum concentration 70%; see 3.5. *Preservation of samples for submission*), or c) placed in freezer at –20°C to avoid further decay and transported frozen.

When testing any population outside an acute mortality event for the presence of crayfish plague, as many individuals as possible should be inspected visually for signs of cuticular damage. Crayfish that have melanized spots or missing limbs should be selected in the first place for further analysis.

3.2. Selection of organs or tissues

In highly susceptible species, the tissue recommended for sampling is the soft abdominal cuticle, which can be found on the ventral side of the abdomen. Any other soft part of the exoskeleton can be included as well. If any melanized spots or whitened areas are detected, these should be included in the sampling. From diseased animals, samples should be aseptically collected from the soft abdominal cuticle. For identification of carriers, samples should be aseptically collected from soft abdominal cuticle, and telson and uropods, separately.

In the North American crayfish species, sampling of soft abdominal cuticle, uropods and telson are recommended. Any other soft part of the exoskeleton can be included as well. If any melanized spots are detected, these should be included in the sampling.

3.3. Samples or tissues not suitable for pathogen detection

Autolysed material is not suitable for analysis.

3.4. Non-lethal sampling

A non-destructive sampling method that detects *A. astaci* DNA in the microbial biofilm associated with the cuticle of individual crayfish through vigorous scrubbing has been described (Pavic *et al.,* 2020), and could be considered in case of testing vulnerable populations.

3.5. Preservation of samples for submission

The use of non-preserved crayfish is preferred, as described above. If transport of recently dead or moribund crayfish cannot be arranged, crayfish may be frozen or fixed in ethanol (minimum 70%). However, fixation may reduce test sensitivity. The crayfish:ethanol ratio should ideally be 1:10 (1 part crayfish, 10 parts ethanol).

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0

3.5.1. Samples for pathogen isolation

The success of pathogen isolation depends strongly on the quality of samples (time since collection and time in storage). Isolation is best attempted from crayfish with clinical signs delivered alive (see Section 3.1.). Fresh specimens should be kept chilled and preferably sent to the laboratory within 24 hours of collection.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

3.5.3. Samples for histopathology

Standard sample collection, preservation and processing methods for histological techniques can be found in Section 2.2 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Sensitive molecular methods can be used to detect *A. astaci* DNA directly from water samples (Strand *et al*. 2011, 2012) These methods require validation for diagnostic use.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger animals should be processed and tested individually. Small life stages such as PL, can be pooled to obtain the minimum amount of material for molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts						+	+	NA				
Histopathology						+	+	NA				
~~Cell~~ Culture						+	+	NA				
Real-time PCR	++	++	++	1	++	++	++	1	++	++	++	1
Conventional PCR	+	+	+	1	++	++	++	1				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation												
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods[3]												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); NA = not available. PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Small pieces of soft cuticle excised from the regions mentioned above (Section 2.3.3 *Gross pathology*) and examined under a compound microscope using low-to-medium power will confirm the presence of aseptate fungal-like hyphae 7–9 µm wide. The hyphae can usually be found pervading the whole thickness of the cuticle, forming a three-dimensional network of hyphae in heavily affected areas of the cuticle. The presence of host haemocytes and possibly some melanisation closely associated with and encapsulating the hyphae give good presumptive evidence that the hyphae represent a pathogen rather than a secondary opportunist invader. In some cases, examination of the surface of such mounted cuticles will demonstrate the presence of characteristic *A. astaci* sporangia with clusters of encysted primary spores (see Section 4.3 *Culture for isolation*).

4.2. Histopathology

Unless the selection of tissue for fixation has been well chosen, *A. astaci* hyphae can be difficult to find in stained preparations. A histological staining technique, such as the Grocott silver stain counterstained with conventional haematoxylin and eosin, can be used. However, such material does not prove that any hyphae observed are those of *A. astaci*, especially when the material comes from animals already dead by sampling.

See also Section 4.1 *Wet mounts*.

4.3. Culture for isolation

Isolation is not recommended as a routine diagnostic method (Alderman & Polglase, 1986; Cerenius *et al*., 1987; Viljamaa-Dirks, 2006). Test sensitivity and specificity of the cultivation method can be very variable depending on the experience of the examiner, but in general will be lower than the PCR. Isolation of *A. astaci* by culture from apparently healthy crayfish is challenging and molecular methods are recommended. A detailed description of this test is available from the Reference Laboratory [5].

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate. Shrimp tissues may be used as negative controls.

Live crayfish can be killed using chloroform, electric current or by mechanical destroying the nerve cords. If live or moribund animals are not available, only recently dead animals should be used for DNA extraction. The soft abdominal cuticle is the preferred sample tissue for DNA extraction. Any superficial contamination should first be removed by thoroughly wiping the soft abdominal cuticle with wet (using autoclaved H_2O) clean disposable swabs. The soft abdominal cuticle is then excised and 30–50 mg ground using a pestle and mortar.

Several PCR assays have been developed with varying levels of sensitivity and specificity. Two assays are described here. Both assays target the ITS (internal transcribed spacer) region of the nuclear ribosomal gene cluster within the *A. astaci* genome.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

4.4.1. Real-time PCR

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling conditions

[5] https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3

Method 1*: Vralstad *et al.,* 2009, Strand, 2013; GenBank Accession No.AM947024			
Aphanomyces astacus & *A. fennicus* ITS	Fwd: AAG-GCT-TGT-GCT-GGG-ATG-TT Rev: CTT-CTT-GCG-AAA-CCT-TCT-GCT-A Probe: 6-FAM-TTC-GGG-ACG-ACC-C-MGBNFQ	500 nM 200 nM	50 cycles of: 95°C/15 sec and 60°C/30 sec
Alternative method 2: Strand *et al*. to be published; GenBank Accession No. AM947024			
Aphanomyces astacus ITS	Fwd: TAT-CCA-CGT-GAA-TGT-ATT-CTT-TAT Rev: GCT-AAG-TTT-ATC-AGT-ATG-TTA-TTT-A Probe: FAM-AAG-AAC-ATC-CCA-GCA-C-MGBNFQ	500 nM 200 nM	50 cycles of: 95°C/15 sec and 60°C/30 sec

*These ITS-based methods have been found to give positive results for the species *Aphanomyces fennicus* (Viljamaa-Dirks & Heinikainen 2019).

The absolute limit of detection of method 1 was reported as approximately 5 PCR forming units (= target template copies), which is equivalent to less than one *A. astaci* genome (Vralstad *et al*., 2009). Another study reported consistent detection down to 50 fg DNA using this assay (Tuffs & Oidtmann, 2011).

Analytical test specificity has been investigated (Tuffs & Oidtmann, 2011; Vralstad *et al*., 2009) and no cross-reaction was observed in these studies. However, a novel species, *Aphanomyces fennicus*, isolated from noble crayfish was reported in 2019 (Viljamaa-Dirks & Heinikainen, 2019) that gave a positive reaction in this test at the same level as *A. astaci*. Due to this problem in specificity, the assay has been modified according to the alternative method 2 (Strand *et al*., manuscript in preparation):

Owing to the repeated discovery of new *Aphanomyces* strains, sequencing is required to determine the species of *Aphanomyces*. In the case of the real-time PCR assay, this requires separate amplification of a PCR product using primers ITS-1 and ITS-4 (see Section 4.5 *Amplicon sequencing*).

4.4.2. Conventional PCR

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling conditions
Method 1*: Oidtmann *et al.,* 2006; GenBank Accession No. AY310499 Product size: 569 bp			
Aphanomyces astacus & *A. fennicus* ITS	Fwd: GCT-TGT-GCT-GAG-GAT-GTT-CT Rev: CTA-TCC-GAC-TCC-GCA-TTC-TG-	500 nM	40 cycles of: 1 min/96°C, 1 min/59°C and 1 min/72°C

*This ITS-based method has been found to give positive results for the species *Aphanomyces fennicus* (Viljamaa-Dirks & Heinikainen 2019).

Confirmation of the identity of the PCR product by sequencing is required as a novel species, *A. fennicus*, isolated from noble crayfish was reported in 2019 (Viljamaa-Dirks & Heinikainen, 2019) that gave a positive reaction in this assay.

The assay consistently detects down to 500 fg of genomic target DNA or the equivalent amount of ten zoospores submitted to the PCR reaction (Tuffs & Oidtmann, 2011).

4.4.3. Other nucleic acid amplification methods

Several genotype-specific molecular methods have been developed that, instead of requiring a pure growth as sample material like the RAPD-PCR assay, can be used to analyse crayfish tissue directly (Di Domenico *et al*., 2021; Grandjean *et al*., 2014; Makkonen *et al.,* 2018; Minardi *et al*., 2018; 2019). Detection of a known genotype group combined with a positive result by a recommended conventional or real-time PCR can be used as a confirmative test in geographical areas where crayfish plague is known to be present. However, the current knowledge of the genotype variation is mostly limited to a few original host species and new genotypes or

subtypes are expected to be found. Thus, the suitability of these methods is limited for initial excluding diagnosis or as confirmative tests in geographical areas not known to be infected.

PCR targeting mitochondrial DNA with *A. astaci* genotype specific primers have been shown to detect the known genotypes of *A. astaci*, but these assays may also provide positive results for some other oomycete genera (Cassabella-Herrero *et al.,* 2021).

4.5. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced, analysed and compared with published sequences.

4.6. *In-situ* hybridisation

Not available.

4.7. Immunohistochemistry

Not available

4.8. Bioassay

No longer used for diagnostic purposes (see Cerenius *et al*., 1988).

4.9. Antibody- or antigen-based detection methods

Not available.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The recommended method for surveillance is real-time PCR, the modified assay by Strand *et al*. (manuscript in preparation).

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [6]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

[6] For example transboundary commodities.

The presence of infection with *Aphanomyces astaci* shall be suspected if at least the following criterion is met:

i. Positive result by real-time PCR
ii. Positive result by conventional PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *Aphanomyces astaci* is considered to be confirmed if the following criterion is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *Aphanomyces astaci* shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Visual observation of hyphae indicative of A. astaci

iii) Observation of hyphae indicative of *A. astaci* in stained histological sections

iv) Culture and isolation of the pathogen

v) Positive result by real-time PCR

vi) Positive result by conventional PCR

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *Aphanomyces astaci* is confirmed if the following criterion is met:

i) Positive result by real-time PCR and positive result by conventional PCR and amplicon sequencing

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *Aphanomyces astaci* are provided in Tables 6.3.1. and 6.3.2 (none available). This information can be used for the design of surveys for infection with *Aphanomyces astaci*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study.

7. References

ALDERMAN D.J. (1996). Geographical spread of bacterial and fungal diseases of crustaceans. *Rev sci. tech. Off. int. Epiz.,* **15**, 603–632.

ALDERMAN D.J. & POLGLASE J.L. (1985). Disinfection for crayfish plague. *Aquacult. Fish. Manage.,* **16**, 203–205.

ALDERMAN D.J. & POLGLASE J.L. (1986). *Aphanomyces astaci:* isolation and culture. *J. Fish Dis*., **9**, 367–379.

ALDERMAN D.J., POLGLASE J.L. & FRAYLING M. (1987). *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *J. Fish Dis*., **10**, 385–393.

CASABELLA-HERRERO G., MARTÍNEZ-RÍOS M., VILJAMAA-DIRKS S., MARTÍN-TORRIJOS L. & DIÉGUEZ-URIBEONDO J. (2021). *Aphanomyces astaci* mtDNA: insights into the pathogen's differentiation and its genetic diversity from other closely related oomycetes. *Fungal Biol.,* **125**, 316–325. doi: 10.1016/j.funbio.2020.11.010. Epub 2020 Dec 2.

CERENIUS L. & SÖDERHÄLL K. (1984). Chemotaxis in *Aphanomyces astaci*, an arthropodparasitic fungus. *J. Invertabr. Pathol.,* **43**, 278–281.

CERENIUS L., SÖDERHÄLL K. & FULLER M.S. (1987). *Aphanomyces astaci* and *Aphanomyces* spp. *In:* Zoosporic fungi in teaching and research, Fuller M.S. & Jaworski A., eds. South-Eastern Publishing Corp., Athens, Georgia, USA. pp 64–65.

CERENIUS L., SÖDERHÄLL K., PERSSON M. & AJAXON R. (1988). The crayfish plague fungus, *Aphanomyces astaci* – diagnosis, isolation and pathobiology. *Freshwater Crayfish*, **7**, 131–144.

DI DOMENICO M., CURINI V., CAPRIOLI R., GIANSANTE C., MRUGAŁA A., MOJŽIŠOVÁ M., CAMMA C. & PETRUSEK A. (2021). Real-Time PCR assays for rapid Identification of common *Aphanomyces astaci* genotypes. *Front. Ecol. Evol.*, **9**, art 597585 doi:10.3389/fevo.2021.597585.

DIEGUEZ-URIBEONDO J., HUANG T.-S., CERENIUS L. & SÖDERHÄLL K. (1995). Physiological adaptation of an *Aphanomyces astaci* strain isolated from the freshwater crayfish *Procambarus clarkii. Mycol. Res*., **99**, 574–578.

GRANDJEAN F., VRÅLSTAD T., DIÉGUEZ-URIBEONDO J., JELIĆ M., MANGOMBI J., DELAUNAY C., FILIPOVÁ L., REZINCIUC S., KOZUBÍKOVÁ-BALCAROVA E., GYONNET D., VILJAMAA-DIRKS S. & PETRUSEK A. (2014). Microsatellite markers for direct genotyping of the crayfish plague pathogen *Aphanomyces astaci* (Oomycetes) from infected host tissues. *Vet. Microbiol*., **170**, 317–324.

HOLDICH D.M., REYNOLDS J.D., SOUTY-GROSSET C. & SIBLEY P.J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowl. Manag. Aquat. Ec.*, **394–395**, 1–46.

HUANG T.S., CERENIUS L. & SÖDERHÄLL K. (1994). Analysis of genetic diversity in the crayfish plague fungus, *Aphanomyces astaci*, by random amplification of polymorphic DNA. *Aquaculture*, **126**, 1–10.

KOZUBIKOVA E., VILJAMAA-DIRKS S., HEINIKAINEN S. & PETRUSEK A. (2011). Spiny-cheek crayfish *Orconectes limosus* carries a novel genotype of the crayfish plague pathogen *Aphanomyces astaci. J. Invertebr. Pathol.,* **108**, 214–216.

MAKKONEN J., JUSSILA J., PANTELEIT J., KELLER N.S., SCHRIMPF A., THEISSINGER K., KORTET R., MARTÍN-TORRIJOS L., SANDOVAL-SIERRA J.V., DIÉGUEZ-URIBEONDO J. & KOKKO H. (2018). MtDNA allows the sensitive detection and haplotyping of the crayfish plague disease agent *Aphanomyces astaci* showing clues about its origin and migration. *Parasitology,* **145**, 1210–1218. https://doi.org/10.1017/S0031182018000227.

MARTIN-TORRIJOS L., CAMPOS LACH M., POU ROVIRA Q. & DIÉGUEZ-URIBEONDO J. (2017). Resistance to the crayfish plague, *Aphanomyces astaci* (Oomycota) in the endangered freshwater crayfish species, *Austropotamobius pallipes*. *PLoS ONE*, **12** (7), e0181226. https://doi.org/10.1371/journal. pone.0181226

MINARDI D., STUDHOLME D.J., OIDTMANN B., PRETTO T. & VAN DER GIEZEN M. (2019). Improved method for genotyping the causative agent of crayfish plague (*Aphanomyces astaci*) based on mitochondrial DNA. *Parasitology*, 146, 1022–1029, doi:10.1017/S0031182019000283

MINARDI D., STUDHOLME D.J., VAN DER GIEZEN M., PRETTO T. & OIDTMANN B. (2018). New genotyping method for the causative agent of crayfish plague (*Aphanomyces astaci*) based on whole genome data. *J. Invertebr. Pathol.*, **156**, 6–13.

OIDTMANN B., GEIGER S., STEINBAUER P., CULAS A. & HOFFMANN R.W. (2006). Detection of *Aphanomyces astaci* in North American crayfish by polymerase chain reaction. *Dis. Aquat. Org.,* **72**, 53–64.

OIDTMANN B., HEITZ E., ROGERS D. & HOFFMANN R.W. (2002). Transmission of crayfish plague. *Dis. Aquat. Org.,* **52**, 159–167.

PAVIC D., ČANKOVIĆ M., PETRIĆ I., MAKKONEN J., HUDINA S., MAGUIRE I., VLADUŠIĆA T., ŠVER L., HRAŠĆANA R., ORLIĆ K., DRAGIČEVIĆ P. & BIELEN A. (2020) Non-destructive method for detecting *Aphanomyces astaci*, the causative agent of crayfish plague, on the individual level. *J. Invertebr. Pathol*., **169**, 107274. https://doi.org/10.1016/j.jip.2019.107274

RENNERFELT E. (1936). Untersuchungen uber die Entwicklung und Biologie des Krebspestpilzes Aphanomyces astaci Schikora. Report of the Institute of Freshwater Research (Drottningholm, Sweden), **10**, 1–21.

SOUTY-GROSSET C., HOLDICH D.M., NOEL P.Y., REYNOLDS J.D. & HAFFNER P. (eds) (2006). Atlas of Crayfish in Europe. Muséum National d'Histoire Naturelle, Paris (Patrimoines naturels, 64), 188 p.

STRAND D.A., HOLST-JENSEN A., VILJUGREIN H., EDVARDSEN B., KLAVENESS D., JUSSILA J. & VRÅLSTAD T. (2011). Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments. *Dis. Aquat. Org*., **95**, 9–17.

STRAND D.A., JUSSILA J., VILJAMAA-DIRKS S., KOKKO H., MAKKONEN J., HOLST-JENSEN A., VILJUGREIN H. & VRÅLSTAD T. (2012). Monitoring the spore dynamics of *Aphanomyces astaci* in the ambient water of latent carrier crayfish. *Vet. Microbiol.,* **160**, 99–107.

STRAND D.A. (2013) Environmental DNA monitoring of the alien crayfish plague pathogen *Aphanomyces astaci* in freshwater systems – Sporulation dynamics, alternative hosts and improved management tools. *Dissertation book, University of Oslo Faculty of Mathematics and Natural Sciences Department of Biosciences, Oslo*, ISSN 1501-7710, 73 p.

SVOBODA J., MRUGAŁA A., KOZUBÍKOVÁ-BALCAROVÁ E. & PETRUSEK A. (2017). Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. *J. Fish Dis*., **40,**127–140. https://doi.org/10.1111/jfd.12472

SODERHALL K. & CERENIUS L. (1999) The crayfish plague fungus: history and recent advances. *Freshwater Crayfish*, **12**, 11-34.

THOMAS J.R., ROBINSON C.V., MRUGAŁA A., ELLISON A.R., MATTHEWS E., GRIFFITHS S.W., CONSUEGRA S. & CABLE J. (2020). Crayfish plague affects juvenile survival and adult behaviour of invasive signal crayfish. *Parasitology*, **1–9**, https://doi.org/10.1017/S0031182020000165

TUFFS S & OIDTMANN B (2011). A comparative study of molecular diagnostic methods designed to detect the crayfish plague pathogen, *Aphanomyces astaci*. *Vet. Microbiol.,* **153**, 345–353.

UNESTAM T. (1966). Studies on the crayfish plague fungus *Aphanomyces astaci*. II. Factors affecting zoospores and zoospore production. *Physiol. Plant*., **19**, 1110–1119.

UNESTAM T. & SODERHALL K. (1977). Specialisation in crayfish defence and fungal aggressiveness upon crayfish plague infection. *Freshwater Crayfish*, **3**, 321–331.

VILJAMAA-DIRKS S. (2006). Improved detection of crayfish plague with a modified isolation method. *Freshwater Crayfish*, **15**, 376–382.

VILJAMAA-DIRKS S. & HEINIKAINEN S. (2019) A tentative new species *Aphanomyces fennicus* sp. nov. interferes with molecular diagnostic methods for crayfish plague. *J. Fish Dis*., **42**, 413–422. https://doi.org/10.1111/jfd.12955

VILJAMAA-DIRKS S., HEINIKAINEN S., NIEMINEN M., VENNERSTRÖM P. & PELKONEN S. (2011). Persistent infection by crayfish plague *Aphanomyces astaci* in a noble crayfish population – a case report. *Bull. Eur. Assoc. Fish Pathol*., **31**, 182–188.

VILJAMAA-DIRKS S., HEINIKAINEN S., TORSSONEN H., PURSIAINEN M., MATTILA J. & PELKONEN S. (2013). Distribution and epidemiology of genotypes of the crayfish plague *Aphanomyces astaci* from noble crayfish *Astacus astacus* in Finland. *Dis. Aquat. Org.*, **103**, 199–208.

VRALSTAD T., JOHNSEN S.I., FRISTAD R., EDSMAN L. & STRAND D.A. (2011). Potent infection reservoir of crayfish plague now permanently established in Norway. *Dis. Aquat. Org.,* **97**, 75–83.

VRALSTAD T., KNUTSEN A.K., TENGS T. & HOLST-JENSEN A. (2009). A quantitative TaqMan® MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague *Aphanomyces astaci*. *Vet. Microbiol.,* **137**, 146–155.

WHITE T.J., BRUNS T., LEE S. & TAYLOR J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* PCR Protocols: A Guide to Methods and Applications, Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., eds. Academic Press, San Diego, California, USA, pp. 315–322.

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NB: There is an OIE Reference Laboratory for infection with *Aphanomyces astaci* (crayfish plague)
(please consult the OIE web site for the most up-to-date list:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the OIE Reference Laboratories for any further information on
infection with *Aphanomyces astaci* (crayfish plague)

NB: FIRST ADOPTED IN 1995; MOST RECENT UPDATES ADOPTED IN 2017.

CHAPTER 2.2.3.

INFECTION WITH HEPATOBACTER PENAEI (NECROTISING HEPATOPANCREATITIS)

1. Scope

~~Infection with infectious salmon anaemia virus (ISAV) means infection with the pathogenic agent highly polymorphic region (HPR)-deleted ISAV, or the non-pathogenic HPR0 (non-deleted HPR) ISAV of the Genus *Isavirus* and Family *Orthomyxoviridae*.~~

Infection with ~~*Candidatus* *Hepatobacter penaei*~~ means infection with the pathogenic agent *Candidatus* H. penaei, an obligate intracellular bacterium of the Family Holosporaceae, Order Rickettsiales ~~α *Proteobacteria*~~.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Hepatobacter penaei is a pleomorphic, Gram-negative, intracytoplasmic bacterium (Nunan *et al.,* 2013). It is a member of the α-*Proteobacteria* (Frelier *et al.,* 1992; Lightner & Redman, 1994; Loy & Frelier, 1996; Loy *et al.,* 1996). More recently it has been suggested that it belongs to the Family Holosporaceae ~~family~~ within the Order Rickettsiales (Leyva *et al.,* 2018). The predominant form is a rod-shaped rickettsial-like organism (0.25 × 0.9 µm), whereas the helical form (0.25 × 2–3.5 µm) possesses eight flagella at the basal apex (Frelier *et al.,* 1992; Lightner & Redman, 1994; Loy & Frelier, 1996; Loy *et al.,* 1996). Genetic analysis of *H. penaei* associated with North and South American outbreaks suggests that the isolates are either identical or very closely related subspecies (Loy *et al.,* 1996). ~~Recently~~ Analysis based on the 16S rRNA confirms the high similarity among different *H. penaei* isolates in the Americas (99–100%) (Aranguren & Dhar, 2018).

2.1.2. Survival and stability in processed or stored samples

Hepatobacter penaei-infected tissues remain infectious after repeated cycles of freeze–thawing and after storage in 50% glycerine. *Hepatobacter penaei* frozen at –20°C to –70°C and –80°C have been shown to retain infectivity in experimental transmission trials with *Penaeus vannamei* (Crabtree *et al.*, 2006; Frelier *et al.,* 1992). Flash freezing *H. penaei* at –70°C to –80°C does not significantly affect the infectivity (Aranguren *et al*., 2010; Crabtree *et al.,* 2006).

2.1.3. Survival and stability outside the host

No information available.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *H. penaei* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) ~~include~~ are: whiteleg shrimp (*P. vannamei*)

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *H. penaei* according to Chapter 1.5. of the *Aquatic Code* ~~include~~ are: aloha prawn (*P. marginatus*), banana prawn

(*P. merguiensis*), blue shrimp (*P. stylirostris*), giant tiger prawn (*P. monodon*), northern brown shrimp (*P. aztecus*), northern pink shrimp (*P. duorarum*) and northern white shrimp (*P. setiferus*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: American lobster (*Homarus americanus*) (Avila-Villa *et al.,* 2012; Bekavac *et al.,* 2022).

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Infection with *H. penaei* has been demonstrated in postlarvae (PL), juveniles, adults and broodstock of *P. vannamei* (Aranguren *et al*., 2006).

2.2.4. Distribution of the pathogen in the host

The target tissue is the hepatopancreas: infection with *H. penaei* has been reported in all hepatopancreatic cell types (Lightner 2012). *Hepatobacter penaei* is also present in the faeces (Brinez *et al.,* 2003).

2.2.5. Aquatic animal reservoirs of infection

Some members of *P. vannamei* populations that survive infection with *H. penaei* may carry the intracellular bacteria for life and transmit it to other populations by horizontal transmission (Aranguren *et al.,* 2006; Lightner, 2005; Morales-Covarrubias, 2010; Vincent & Lotz, 2005).

2.2.6. Vectors

No vectors are known in natural infections.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Infection with *H. penaei* often causes an acute disease with very high mortalities in young juveniles, adults and broodstock. In horizontally infected young juveniles, adults and broodstock, the incubation period and severity of the disease are somewhat size or age dependent. Infection with *H. penaei* results in the mortalities approaching 100% in *P. vannamei*, 5.6–15% in *P. duorarum*, and 5–17% in *P. aztecus* (Aguirre-Guzman *et al.,* 2010).

The prevalence was reported as 0.77% in cultured *P. vannamei* and 0.43% in cultured *P. stylirostris* in Peru (Lightner & Redman, 1994), 5–86.2% in Mexico (Ibarra-Gamez *et al.,* 2007), and 0.6–1.3% in *P. vannamei* in Belize, Brazil, Guatemala, Honduras, Mexico, Nicaragua and Venezuela (Morales-Covarrubias *et al*., 2011).

NHP-affected broodstock ponds in Colombia reported mortalities of up to 85%, while non NHP-affected broodstock ponds in the same farm experienced mortalities of 40–50% (Aranguren *et al.,* 2006).

2.3.2. Clinical signs, including behavioural changes

A wide range of gross signs can be used to indicate the possible presence of infection with *H. penaei*. These include lethargy, reduced food intake, atrophied hepatopancreas, anorexia and empty guts, noticeably reduced growth and poor length weight ratios ('thin tails'); soft shells and flaccid bodies; black or darkened gills; heavy surface fouling by epicomensals organisms; bacterial shell disease, including ulcerative cuticle lesions or melanised appendage erosion; and expanded chromatophores resulting in the appearance of darkened edges in uropods and pleopods. None of these signs are pathognomonic. (Lightner, 1996; Loy *et al.,* 1996).

2.3.3 Gross pathology

Infection with *H. penaei* often causes an acute disease with very high mortalities in young juveniles, adults and broodstock. In horizontally infected young juveniles, adult and broodstock, the incubation period and severity of the disease are somewhat size or age dependent. Gross signs are not specific, but shrimp with acute infection with *H. penaei* show atrophied hepatopancreas, empty guts, soft shells and flaccid bodies; black or darkened gills; bacterial shell disease, including ulcerative cuticle lesions or melanised appendage erosion; and expanded chromatophores resulting in the appearance of darkened edges in uropods and pleopods. None of these signs are pathognomonic. (Lightner, 1996; Loy *et al.,* 1996) a marked reduction in food consumption, followed by

~~changes in behaviour and appearance including pale discoloration of the hepatopancreas with further size reduction~~.

2.3.4. Modes of transmission and life cycle

Horizontal transmission of *H. penaei* can be through cannibalism or by contaminated water (Aranguren *et al.,* 2006; 2010; Frelier *et al.,* 1993; Gracia-Valenzuela *et al.*, 2011; Vincent *et al.*, 2004). *Hepatobacter penaei* in faeces shed into pond water has also been suggested as a source of contamination (Aranguren *et al.,* 2006; Briñez *et al.,* 2003; Morales-Covarrubias *et al.*, 2006). *Hepatobacter penaei*-positive broodstock females produce PL that were also *H. penaei*-positive, which suggests that a transmission from broodstock to progeny can occur~~s~~ (Aranguren *et al.,* 2006).

2.3.5. Environmental factors

The occurrence of infection with *H. penaei* in farms may increase during long periods of high temperatures (>29°C) and high salinity (20–38 ppt) (Morales-Covarrubias, 2010). In the months when temperatures are high during the day and low at night, high prevalence and mortality (>20%) are observed (Morales-Covarrubias, 2010).

2.3.6. Geographical distribution

Hepatobacter penaei appears to have a Western Hemisphere distribution in both wild and cultured penaeid shrimp (Aguirre-Guzman *et al.,* 2010; Del Rio-Rodriguez *et al.,* 2006). In the Western Hemisphere, *H. penaei* is commonly found in cultured penaeid shrimp in the Americas (Aranguren *et al.,* 2010; Frelier *et al.,* 1992; Ibarra-Gamez *et al.,* 2007; Morales-Covarrubias, 2010; Morales-Covarrubias *et al.*, 2011). *Hepatobacter penaei*, was introduced into Africa from North America via movement of infected *P.vannamei* broodstock, however NHP was later eradicated by fallowing (Lightner *et al.,* 2012).

See OIE WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

Early detection (initial phase) of clinical infection with *H. penaei* is important for successful treatment because of the potential for cannibalism to amplify and transmit the disease. Shrimp starvation and cannibalism of infected shrimp, and positive conditions for *H. penaei* ~~enaei~~ multiplication, are important factors for the spread of *H. penaei* in *P. vannamei*. Preventive measures include raking, tilling, and removing sediments from the bottom of the ponds, prolonged drying (through exposure to sunlight) of ponds and water distribution canals for several weeks, disinfection of fishing gear and other farm equipment using calcium hypochlorite, extensive liming of ponds and the use of ponds liners. The use of specific pathogen-free (SPF) broodstock is an effective preventive measure. NHP, particularly in the initial phase, can be treated by using antibiotics in medicated feeds. ~~*Hepatobacter penaei* is sensitive to oxytetracycline (Lightner & Redman, 1994).~~

2.4.1. Vaccination

No scientifically confirmed reports.

2.4.2. Chemotherapy including blocking agents

No scientifically confirmed reports.

2.4.3. Immunostimulation

No scientifically confirmed reports.

2.4.4. Breeding resistant strains

One population from Latin America that has been selected for several generations for resistance to Taura syndrome virus in the presence of infection with *H. penaei*, seems to be more resistant to NHP disease than the Kona line under experimental conditions (Aranguren *et al.,* 2010).

2.4.5. Inactivation methods

The use of hydrated lime ($Ca(OH)_2$) to treat the bottom of ponds during pond preparation before stocking can help reduce infection with *H. penaei*.

2.4.6. Disinfection of eggs and larvae

Disinfection of eggs and larvae is a good management practice and is recommended for its potential to reduce *H. penaei* contamination of spawned eggs and larvae (and contamination by other disease agents).

2.4.7. General husbandry

The prevalence and severity of infection with *H. penaei* may be increased by rearing shrimp in relatively crowded or stressful conditions. Some husbandry practices have been successfully applied to the prevention of infection with *H. penaei*. Among these has been the application of PCR to pre-screening of wild or pond-reared broodstock.

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

Suitable specimens for testing for infection with *H. penaei* are the following life stages: PL, juveniles and adults.

3.2. Selection of organs or tissues

Hepatobacter penaei infects most enteric tissue. The principal target tissue for *H. penaei* is the hepatopancreas and this organ should be selected preferentially (Lightner, 2012).

3.3. Samples or tissues not suitable for pathogen detection

Hepatobacter penaei does not replicate in the midgut, caeca, connective tissue cells, the gills, haematopoietic nodules and haemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, and lymphoid organ parenchymal cells. Samples of pleopods or haemolymph are not recommended for *H. penaei* detection by PCR.

3.4. Non-lethal sampling

Hepatobacter penaei can be detected in faeces samples collected from clinically affected populations of *Penaeus vannamei* ~~may be collected and used for testing (usually by PCR),~~ when non-lethal testing of ~~valuable~~ broodstock is necessary (Brinez *et al.,* 2003~~; Frelier *et al.,* 1993; Lightner, 1996~~). However, the use of faeces samples to detect NHP in apparently healthy shrimp has not been evaluated. ~~Faeces samples have not been validated to the same level as hepatopancreas samples.~~

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (*diseases of crustaceans*)

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternate storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples of hepatopancreas or faeces for PCR testing should be preserved in 70–95% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen, but repeated freezing and thawing should be avoided.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section 5.3 of Chapter 2.2.0.

3.5.4. Samples for other tests

No scientifically confirmed reports.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger shrimp should be processed and tested individually. Small life stages such as PL or specimens up to 0.5 g can be pooled to obtain the minimum amount of material for *H. penaei* molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts						+	+	NA				
Histopathology						++	++	NA				
Cell culture												
Real-time PCR	++	+++	+++	1	++	+++	+++	1	++	++	++	1
Conventional PCR	++	++ ~~+~~	++ ~~+~~	1	++	+++	+++	1	~~++~~	~~+++~~	~~+++~~	~~1~~
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
***In-situ* hybridisation**					+	++	++	NA	+	++	++	NA
Bioassay					+	+	+	NA	+	+	+	NA
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods[3]												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); NA = not available; PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

[3]~~Specify the test used.~~ Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Wet mount squash examination of hepatopancreas tissue is generally conducted to detect presumptive infection with *H. penaei*. The hepatopancreas may be atrophied and have any of the following characteristics: soft and watery; fluid filled centre; pale colour with or without black stripes (melanised tubules). Hepatopancreatic tubules show deformity at the distal portion; multifocal melanisation initially at the distal portion of the tubule and, later on, in the medial and proximal portion; reduced or absence of lipid droplets (Lightner, 2012).

4.2. Histopathology and cytopathology

Histological methods can be useful for indicating acute and chronic infection with *H. penaei*.

Initial infection with *H. penaei* is difficult to diagnose using routine H&E histological methods. Therefore, molecular methods are recommended for initial *H. penaei* detection (e.g. by PCR or application of *H. penaei*-specific DNA probes or *in-situ* hybridisation ~~[ISH]~~ of histological sections).

Acute infection with *H. penaei* is characterised by atrophied hepatopancreas with moderate atrophy of the tubule epithelia, presence of bacterial cells and infiltrating haemocytes involving one or more of the tubules (multifocal encapsulations). Hypertrophic cells, individual epithelial cells, appeared to be separated from adjacent cells, undergo necrosis and desquamation into the tubular lumen. The tubular epithelial cell lipid content is variable.

The transitional phase of infection with *H. penaei* is characterised by haemocytic inflammation of the intertubular spaces in response to necrosis, cytolysis, and sloughing of hepatopancreas tubule epithelial cells. The hepatopancreas tubule epithelium is markedly atrophied, resulting in the formation of large oedematous (fluid filled or 'watery') areas in the hepatopancreas. Tubule epithelial cells within multifocal encapsulation are typically atrophied and reduced from simple columnar to cuboidal morphology. They contain little or no stored lipid vacuoles, markedly reduced or no secretory vacuoles and masses of bacteria. At this phase haemocyte nodules are observed in the presence of masses of bacteria in the centre of the nodule.

In the chronic phase of infection with *H. penaei*, tubular lesions, multifocal encapsulation and oedematous areas decline in abundance and severity and are replaced by infiltration and accumulation of haemocytes at the sites of necrosis. There are areas with fibrosis, few melanised and necrotic tubules and very low presence of hypertrophied cells with masses of bacteria in the cytoplasm and low numbers of haemocyte nodules.

4.3. Cell culture for isolation

Hepatobacter penaei has not been grown *in vitro*. No crustacean cell lines exist (Vincent & Lotz, 2007).

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

PCR methods including PCR and real-time PCR have been developed that target several *H. penaei* genes including 16S rRNA and Flg E genes (Aranguren & Dhar, 2018; Aranguren *et al.,* 2010; Loy *et al*., 1996).

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

~~*DNA extraction*~~

~~A general DNA extraction method may be used to extract DNA from the hepatopancreatic tissue of putatively infected shrimp. The amount of template DNA in a 10–25 µl PCR reaction volume should be in the range of 10–100 ng of total DNA~~

4.4.1. Real-time PCR

Real-time PCR methods for detection of *H. penaei* have the advantages of speed, specificity and sensitivity. The sensitivity of real-time PCR is ~100 copies of the target sequence from the *H. penaei* genome (Aranguren & Dhar, 2018; Aranguren *et al.,* 2010; Vincent & Lotz, 2005).

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Aranguren *et al.,* 2010; GenBank U65509			
16S rRNA gene	Fwd NHP1300F: CGT-TCA-CGG-GCC-TTG-TAC-AC Rev NHP1366R: GCT-CAT-CGC-CTT-AAA-GAA-AAG-ATA-A Probe: CCG-CCC-GTC-AAG-CCA-TGG-AA	300 nM 100 nM	40 cycles: 95°C/15 sec and 60°C/1 min
Method 2: Aranguren & Dhar 2018; GenBank JQAJ01000001.1			
Flagella hook protein	Fwd NHP FlgE3qF: AAC-ACC-CTG-TCT-CCC-CAA-TTC Rev FlgE3qR: CCA-GCC-TTG-GAC-AAA-CAC-CTT Probe: CGC-CCC-AAA-GCA-TGC-CGC	500 nM 100 nM	40 cycles: 95°C/1 sec and 60°C/20 sec

~~**Protocol 1**~~

~~The real-time PCR method using TaqMan chemistry described below for *H. penaei* based on the 16S rRNA gene generally follows the method used in Aranguren *et al.* (2010).~~

~~i) The PCR primers and TaqMan probe are selected from the 16S, rRNA gene of *H. penaei* (GenBank U65509) (Loy & Frelier, 1996). The primers and TaqMan probe were designed by the Primer Express software version 2.0 (Applied Biosystems). The upstream (NHP1300F) and downstream (NHP1366R) primer sequences are: 5' CGT TCA CGG GCC TTG TACAC 3' and 5' GCT CAT CGC CTT AAA GAA AAG ATA-A-3', respectively. The TaqMan probe NHP: 5'-CCG-CCC-GTC-AAG-CCA-TGG-AA-3', which corresponds to the region from nucleotides 1321–1340, is synthesised and labelled with fluorescent dyes 6-carboxyfluorescein (FAM) on the 5' and N,N,N,Ntetramethyl-6-carboxyrhodamine (TAMRA) on the 3' end.~~

~~ii) *The real-time PCR reaction mixture contains:* TaqMan One step real-time PCR SuperMix (Quanta, Biosciences), 0.3 µM of each primer, 0.1 µM of TaqMan probe, 5–50 ng DNA, and water in a reaction volume of 25 µl. For optimal results, the reaction mixture should be vortexed and mixed well.~~

~~iii) Amplification is performed with the master cycler Realplex 2.0 (Eppendorf). The cycling consists of initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. After each cycle, the levels of fluorescence are measured.~~

~~iv) It is necessary to include a 'no template control' in each reaction run. This is to rule out the presence of fluorescence contaminants in the reaction mixture and also to rule out reagent contamination with the specific target of the assay. A positive control should also be included, and this can be plasmid DNA containing the target sequence, purified bacteria, or DNA extracted from *H. penaei*-infected hepatopancreas.~~

~~**Protocol 2**~~

~~Another real-time PCR method using TaqMan chemistry described below for *H. penaei* is based on the flagella gene (flagella hook protein, flgE) (Aranguren & Dhar, 2018).~~

~~i) The PCR primers and TaqMan probe were selected from the Flg E gene of *H. penaei* (GenBank JQAJ01000001.1) (Aranguren & Dhar, 2018). The primers and TaqMan probe were designed by the Primer Express software version 3.0 (Applied Biosystems). The upstream (NHP FlgE3qF) and downstream (FlgE3qR) primer sequences are: 5'-AAC-ACC-CTG-TCT-CCC-CAA-TTC-3'; and 5'-CCA-GCC-TTG-GAC-AAA-CAC-CTT-3', respectively. The TaqMan probe NHP: 5'-CGC-CCC-AAA-GCA-TGC-CGC-3', is synthesised and labelled with fluorescent dyes 6-carboxyfluorescein (FAM) on the 5' and N,N,N,Ntetramethyl-6-carboxyrhodamine (TAMRA) on the 3' end.~~

~~ii) *The real-time PCR reaction mixture contains:* The amplification reactions were conducted as follows: 0.5 µM of each primer, 0.1 µM TaqMan probe, 1× TaqMan Fast Virus 1-Step Master Mix (Life Technologies), 5–~~

~~50 ng DNA template and HPLC water in a reaction volume of 10 µl. For optimal results, the reaction mixture should be vortexed and mixed well.~~

iii) ~~The real-time PCR profile consists of 20 seconds at 95°C followed by 40 cycles of 1 second at 95°C and 20 seconds at 60°C. Amplification detection and data analysis for real-time PCR assays are carried out with the StepOnePlus real-time PCR system (Life Technologies).~~

iv) ~~It is necessary to include a 'no template control' in each reaction run. This is to rule out the presence of fluorescence contaminants in the reaction mixture and also to rule out reagent contamination with the specific target of the assay. A positive control should also be included, and this can be plasmid DNA containing the target sequence, or DNA extracted from *H. penaei* infected hepatopancreas.~~

4.4.2. Conventional PCR

Hepatopancreas may be assayed for *H. penaei* using PCR. Two different PCR methods have been developed for *H. penaei* detection using 16S rRNA gene and Flg E gene separately.

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Aranguren *et al.*, 2010; GenBank: MH230908.1; 379 bp			
16S rRNA gene	Fwd NHPF2: CGT-TGG-AGG-TTC-GTC-CTT-CAG-T Rev NHPR2: GCC-ATG-AGG-ACC-TGA-CAT-CAT-C	200 nM	35 cycles: 95°C/30 sec, 60°C/30 sec and 72°C/30 sec
Method 2: Aranguren & Dhar, 2018; JQAJ01000001.1; 333 bp			
Flagella hook protein	Fwd FlgE 1143F: AGG-CAA-ACA-AAC-CCT-TG Rev FlgE 1475R: GCG-TTG-GGA-AAG-TT	0.2 µM	35 cycles,: 95°C for 30 sec, 62°C for 30 sec, and 72°C for 30 sec

~~**Protocol 1**~~

~~The PCR based on 16S rRNA is based on Aranguren *et al.* (2010). Primers designated as NHPF2: 5'-CGT-TGG-AGG-TTC-GTC-CTT-CAGT-3' and NHPR2: 5'-GCC-ATG-AGG-ACC-TGA-CAT-CAT-C-3', amplify a 379-base pair (bp) fragment corresponding to the 16S rRNA of *H. penaei*. The PCR method outlined below generally follows the method described in Aranguren *et al.* (2010).~~

i) ~~The following controls should be included when performing the PCR assay a) known *H. penaei* negative tissue sample; b) a known *H. penaei* positive sample (hepatopancreas); and c) a 'no template' control.~~

ii) ~~The PuReTaq™ Ready-To-Go PCR Bead (RTG beads, GE Healthcare) is used for all amplification reactions described here.~~

iii) ~~The optimised PCR conditions (5–50 ng DNA) (final concentrations in 25 µl total volume) for detection of *H. penaei* in shrimp hepatopancreas samples are: primers (0.2 µM each), dNTPs (200 µM each), Taq polymerase (0.1 U μl^{-1}), magnesium chloride (1.5 mM) in 10 mM Tris-HCl, pH 9.0, 50 mM KCl.~~

iv) ~~The cycling parameters are: Step 1: 95°C for 5 minutes, 1 cycle; Step 2: 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds, 35 cycles; Step 3: 60°C for 1 minute, 72°C for 2 minutes, 1 cycle; 4°C infinite hold.~~

~~**Protocol 2**~~

~~The PCR based on flagella gene (flagella hook protein, flgE) is based on Aranguren & Dhar (2018). Primers designated as NHP FlgE 1143F (5'-AGG CAA ACA AAC CCT TG-3') and and the NHP FlgE 1475R (5'- GCG TTG GGA AAG TT-3') amplify a 333-base pair (bp) fragment corresponding to the Flg E of *H. penaei*.~~

i) ~~The following controls should be included when performing the PCR assay a) known *H. penaei* negative tissue sample; b) a known *H. penaei* positive sample (hepatopancreas); and c) a 'no template' control.~~

~~ii) The PuReTaq™ Ready-To-Go PCR Bead (RTG beads, GE Healthcare) is used for all amplification reactions described here.~~

~~iii) The optimised PCR conditions (5–50 ng DNA) (final concentrations in 25 µl total volume) for detection of *H. penaei* in shrimp hepatopancreas samples are: primers (0.2 µM each), dNTPs (200 µM each), Taq polymerase (0.1 U μl^{-1}), magnesium chloride (1.5 mM) in 10 mM Tris-HCl, pH 9.0, 50 mM KCl.~~

~~iv) The cycling parameters are: initial denaturation at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 5 minutes followed by 4°C infinite hold.~~

~~Note: The conditions should be optimised for each thermal cycler using known positive controls.~~

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~

4.4.3. Other nucleic acid amplification methods

None.

4.5. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced, analysed and compared with published sequences.

~~PCR products may be cloned and sequenced or sequenced directly when necessary to confirm infection with *H. penaei* or to identify false positives or nonspecific amplification (Aranguren *et al.*, 2010; Aranguren & Dhar, 2018; Vincent & Lotz, 2005).~~

4.6. *In-situ* hybridisation

The ISH method of Loy & Frelier (1996) and Lightner (1996) provides greater diagnostic sensitivity than do more traditional methods for *H. penaei* detection and diagnosis of infection that employ classical histological methods (Lightner, 1996; Morales-Covarrubias, 2010). The ISH assay of routine histological sections of acute, transition and chronic phase lesions in hepatopancreas with a specific DIG-labelled DNA probe to *H. penaei* 16S rRNA provides a definitive diagnosis of infection with *H. penaei* (Lightner, 1996; Loy & Frelier, 1996; Morales-Covarrubias *et al.,* 2006). Pathognomonic *H. penaei* positive lesions display prominent blue to blue-black areas in the cytoplasm of affected cells when reacted with the DNA probes. (See Chapter 2.2.4 *Infection with infectious hypodermal and haematopoietic necrosis virus* for details of the ISH method, and Chapter 2.2.0 Section B.5.3.ii for detailed information on the use of Davidson's AFA fixative.)

4.7. Immunohistochemistry

Immunohistochemistry (IHC) tests using monoclonal antibodies (MAbs) to *H. penaei*, according to the methods described in Bradley-Dunlop *et al.* (2004), ~~are available~~ exist for *H. penaei* detection.

4.8. Bioassay

Confirmation of infection with *H. penaei* may be accomplished by bioassay of suspect animals with SPF juvenile *P. vannamei* serving as the indicator of the intracellular bacteria (Aranguren *et al.,* 2010; Lightner, 2005). Oral protocols may be used. The oral method is relatively simple to perform and is accomplished by feeding chopped hepatopancreas of suspect shrimp to SPF juvenile *P. vannamei* in small tanks. The use of a negative control tank of indicator shrimp, which receive only a normal feed, is required. When the hepatopancreas feeding (*per os*) protocol is used to bioassay for *H. penaei*, *positive* indicator shrimp (by gross signs and histopathology) are typically apparent within 3–4 days of initial exposure, and significant mortalities occur by 3–8 days after initial exposure. The negative control shrimp must remain negative (for at least 10–15 days) for gross or histological signs of infection with *H. penaei* and unusual mortalities.

4.9. Antibody- or antigen-based detection methods

Serological tests are not applicable because shrimp are invertebrate animals that do not produce specific antibodies that could be used to demonstrate infection by or prior exposure to *H. penaei*.

4.10. Other methods

No scientifically confirmed reports.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR ~~are~~ is the recommended test for surveillance to demonstrate freedom from infection with *H. penaei* in apparently healthy populations as described in Section 4.4.1 ~~and 4.4.2~~.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [7]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical ~~Geographical~~ proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *H. penaei* shall be suspected if at least one of the following criteria is met:

i) A positive result by real-time PCR

ii) A positive result by conventional PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *H. penaei* is considered to be confirmed if at least one of the following criteria is met:

i) A positive result by two different probe-based real-time PCR tests targeting different region of the *H. penaei* genome

ii) A positive result by real-time PCR and conventional PCR targeting different region of the *H. penaei* genome followed by amplicon sequencing

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.2. Clinically affected animals

[7] For example transboundary commodities.

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *H. penaei* shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs consistent with *H. penaei* infection

ii) Histopathology consistent with *H. penaei* infection

iii) A positive result by real-time PCR

iv) A positive result by conventional PCR

v) A positive result by *in-situ* hybridisation

vi) A positive result by bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *H. penaei* is considered to be confirmed if at least at least one of the following criteria is met:

i) A positive result by two different probe-based real-time PCR tests targeting different regions of the *H. penaei* genome

ii) A positive result by real-time PCR and conventional PCR targeting different regions of the *H. penaei* genome followed by amplicon sequencing

iii) ~~Histopathology consistent with *H. penaei* and positive *in-situ* hybridisation test~~ A positive result by *in-situ* hybridisation and real-time PCR

iv) A positive result by *in-situ* hybridisation and conventional PCR followed by amplicon sequencing

v) A positive result by bioassay followed by real-time PCR

vi) A positive result by bioassay followed by conventional PCR followed by amplicon sequencing

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *H. penaei* are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with *H. penaei*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
PCR: = polymerase chain reaction, ND = Not determined.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study, PCR: = polymerase chain reaction.

7. References

AGUIRRE-GUZMAN G., SANCHEZ-MARTINEZ J.G., PÉREZ-CASTAÑEDA R. & ORTA-RODRIGUEZ R. (2010). Detection of necrotizing hepatopancreatitis (NHP) in wild shrimp from Laguna Madre, Mexico by a multiplex polymerase chain reaction. *Thai J. Vet. Med.*, **40**, 337–341.

ARANGUREN L.F., BRIÑEZ B., ARAGON L., PLATZ C., CARABALLO X., SUAREZ A. & SALAZAR M. (2006). Necrotizing hepatopancreatitis (NHP) infected *Penaeus vannamei* female broodstock: effect on reproductive parameters nauplii and larvae quality. *Aquaculture*, **258**, 337–343.

ARANGUREN L.F. & DHAR ARUN K. (2018). Detection and quantification of *Hepatobacter penaei* bacteria (NHPB) by new PCR and real-time quantitative PCR assays. *Dis. Aquat. Org.*, **131**,: 49–57.

ARANGUREN L.F., TANG K.F.J. & LIGHTNER D.V. (2010). Quantification of the bacterial agent of necrotizing hepatopancreatitis (NHP-B) by real-time PCR and comparison of survival and NHP load of two shrimp populations. *Aquaculture*, **307**, 187–192.

AVILA-VILLA L.A., GOLLAS-GALVAN T., MARTINEZ-PORCHAS M., MENDOZA-CANO F. & HERNANDEZ-LOPEZ J. (2012). Experimental infection and detection of necrotizing hepatopancreatitis bacterium in the American lobster *Homarus americanus*. *Sci. World J.*, **2012**, 979381, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3356760/

BEKAVAC A., BECK A., DRAGIČEVIĆ P., DRAGUN Z., MAGUIRE I., IVANKOVIĆ D., FIKET Ž., GRAČAN R. , HUDINA S. (2022). Disturbance in invasion? Idiopathic necrotizing hepatopancreatitis in the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) in Croatia. *J. Fish Dis.*, **45**, 261–276.

BRADLEY-DUNLOP D.J., PANTOJA C. & LIGHTNER. D.V. (2004). Development of monoclonal antibodies for detection of necrotizing hepatopancreatitis in penaeid shrimp. *Dis. Aquat. Org.*, **60**, 233–240.

BRINEZ B., ARANGUREN F. & SALAZAR M. (2003). Fecal samples as DNA source for the diagnosis of necrotizing hepatopancreatitis (NHP) in *Penaeus vannamei* broodstock. *Dis. Aquat. Org.*, **55**, 69–72.

CRABTREE B.G., ERDMAN M.M., HARRIS D.L. & HARRIS I.T. (2006). Preservation of necrotizing hepatopancreatitis bacterium (NHPB) by freezing tissue collected from experimentally infected *Litopenaeus vannamei*. *Dis. Aquat. Org.*, **70**, 175–179.

DEL RÍO-RODRIGUEZ R.E., SOTO-RODRÍGUEZ S., LARA-FLORES M., CU-ESCAMILLA A.D. & GOMEZ-SOLANO M.I. (2006). A necrotizing hepatopancreatitis (NHP) outbreak in a shrimp farm in Campeche, Mexico: A first case report. *Aquaculture*, **255**, 606–609.

FRELIER P.F., LOY J.K. & KRUPPENBACH B. (1993). Transmission of necrotizing hepatopancreatitis in *Penaeus vannamei*. *J. Invertebr. Pathol,* **61**, 44–48.

FRELIER P.F., SIS R.F., BELL T.A. & LEWIS D.H. (1992). Microscopic and ultrastructural studies of necrotizing hepatopancreatitis in Pacific white shrimp (*Penaeus vannamei*) cultured in Texas. *Vet. Pathol.*, **29**, 269–277.

GRACIA-VALENZUELA M.H., LUZ ANGELICA ÁVILA-VILLA L.A., GLORIA YEPIZ-PLASCENCIA G., HERNÁNDEZ-LÓPEZ J., MENDOZA-CANO F., GARCÍA-SANCHEZ G. & GOLLAS-GALVÁN T. (2011). Assessing the viability of necrotizing hepatopancreatitis bacterium (NHPB) stored at –20°C for use in forced-feeding infection of *Penaeus* (*Litopenaeus*) *vannamei*. *Aquaculture*, **311**, 105–109.

IBARRA-GAMEZ J.C., GALAVÍZ-SILVA L. & MOLINA-GARZA Z.J. (2007). Distribution of necrotizing hepatopancreatitis bacterium (NHPB) in cultured white shrimp, *Litopenaeus vannamei*, from Mexico. *Cienc. Mar.,* **33,** 1–9.

KROL R.M., HAWKINS W.E. & OVERSTREET R.M. (1991). Rickettsial and mollicute infections in hepatopancreatic cells of cultured pacific white shrimp (*Penaeus vannamei*). *J. Invertebr. Pathol.*, **57**, 362–370.

LEYVA J.M., MARTINEZ-PORCHAS M., HERNANDEZ-LOPEZ J., VARGAS-ALBORES F.& T. GOLLAS-GALVAN (2018). Identifying the causal agent of necrotizing hepatopancreatitis in shrimp: Multilocus sequence analysis approach *Aquaculture Res*., 1–8.

LIGHTNER D.V (ed.) (1996). A handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, LA, USA, 304 p.

LIGHTNER D.V. (2005). Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *J. World Aquac. Soc*., **36**, 229–248.

LIGHTNER D.V. & REDMAN R.M. (1994). An epizootic of necrotizing hepatopancreatitis in cultured penaeid shrimp (Crustacea: Decapoda) in northwestern Peru. *Aquaculture*, **122**, 9–18.

LIGHTNER D.V., REDMAN R.M., PANTOJA C.R., TANG K.F.J., NOBLE B.L., SCHOFIELD P., MOHNEY L.L., NUNAN L.M. & NAVARRO S.A. (2012). Historic emergence, impact and current status of shrimp pathogens in the Americas. *J. Invertebr. Pathol*., **110**, 174–183.

LOY J.K., DEWHIRST F.E., WEBER W., FRELIER P.F., GARBAR T.L., TASCA S.I & TEMPLETON J.W. (1996). Molecular phylogeny and *in situ* detection of the etiologic agent of necrotizing hepatopancreatitis in shrimp. *Appl. Environ. Microbiol*., **62**, 3439–3445.

LOY J.K. & FRELIER P.F. (1996). Specific, nonradioactive detection of the NHP bacterium in *Penaeus vannamei* by *in situ* hybridization. *J. Vet. Diagn. Invest.,* **8**, 324–331.

LOY J.K., FRELIER P.F., VARNER P. & TEMPLETON J.W. (1996). Detection of the etiologic agent of necrotizing hepatopancreatitis in cultured *Penaeus vannamei* from Texas and Peru by polymerase chain reaction. *Dis. Aquat. Org*., **25**, 117–122.

MORALES-COVARRUBIAS M.S. (2010). Enfermedades del camarón. Detección mediante análisis en fresco e histopatología. Editorial Trillas, SA de CV., Av. Río Churubusco 385, Col. Pedro María Anaya, México, D.F. Segunda edición. ISBN: ISBN 978-607-17-0436-8. 1-180.

MORALES-COVARRUBIAS M.S., OSUNA-DUARTE A.G., GARCIA-GASCA A., LIGHTNER D.V. & MOTA-URBINA J.C. (2006). Prevalence of necrotizing hepatopancreatitis in female broodstock of *Penaeus vannamei* with unilateral eyestalk ablation and hormone injection. *J. Aquat. Anim. Health*, **18**, 19–25.

MORALES-COVARRUBIAS M.S., RUIZ-LUNA A., MOURA-LEMUS A.P., SOLÍS MONTIEL V.T. & CONROY G. (2011). Prevalencia de enfermedades de camarón blanco (*Litopenaeus vannamei*) cultivado en ocho regiones de latinoamérica. *Rev. Cient. (Maracaibo)*, **XXI**, 434–446.

NUNAN L.M., PANTOJA C.R., GOMEZ-JIMENEZ S. & LIGHTNER D.V. (2013). "*Candidatus* Hepatobacter penaei," an intracellular pathogenic enteric bacterium in the hepatopancreas of the marine shrimp *Penaeus vannamei* (Crustacea: Decapoda). *Appl. Environ. Microbiol*., **79**, 1407–1409.

VINCENT A.G., BRELAND V.M. & LOTZ J.M. (2004). Experimental infection of Pacific white shrimp *Litopenaeus vannamei* with necrotizing hepatopancreatitis (NHP) bacterium by *per os* exposure. *Dis. Aquat. Org.,* **61**, 227–233

VINCENT A.G. & LOTZ J.M. (2005). Time course of necrotizing hepatopancreatitis (NHP) in experimentally infected *Litopenaeus vannamei* and quantification of NHP-bacterium using real-time PCR. *Dis. Aquat. Org.,* **67**, 163–169.

VINCENT A.G. & LOTZ J.M. (2007). Effect of salinity on transmission of necrotizing hepatopancreatitis bacterium (NHPB) to Kona stock *Litopenaeus vannamei. Dis. Aquat. Org.,* **75**, 265–268.

*
* *

NB: There is an OIE Reference Laboratory for infection with *Hepatobacter penaei*
(necrotising hepatopancreatitis)
(please consult the OIE web site for the most up-to-date list:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the OIE Reference Laboratories for any further information on
infection with *Hepatobacter penaei* (necrotising hepatopancreatitis).

NB: FIRST ADOPTED IN 2012; MOST RECENT UPDATES ADOPTED IN 2017.

CHAPTER 2.2.4.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

Infection with infectious hypodermal and haematopoietic necrosis virus means *infection* with the *pathogenic agent Decapod penstylhamaparvovirus* 1, of the Genus *Penstylhamaparvovirus* and Family *Parvoviridae* ~~infection with the pathogenic agent infectious hypodermal and haematopoietic necrosis virus (IHHNV), Family *Parvoviridae*, subfamily *Hamaparvovirinae*,~~ Genus *Penstylhamaparvovirus* ~~with IHHNV (*Decapod penstylhamaparvovirus 1)* as the Type species (Penez *et al.*, 2020)~~.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

IHHNV is the smallest of the known penaeid shrimp viruses. The virion is a 20–22 nm, non-enveloped icosahedron, with a density of 1.40 g ml^{-1} in CsCl that contains linear single-stranded DNA with an estimated size of 3.9 kb (GenBank NC_002190), and has a capsid with four polypeptides of molecular weight 74, 47, 39, and 37.5 kD (Bonami *et al.,* 1990; Nunan *et al.,* 2000~~; GenBank NC_002190~~) .

At least two distinct genotypes of IHHNV have been identified (Tang *et al.,* 2003): Type 1 is from the Americas and South-East Asia (principally the Philippines) and Type 2 is from South-East Asia. These genotypes were shown to be ~~are~~ infectious to *Penaeus vannamei* and *P. monodon* (Tang *et al.,* 2003). Two sequences homologous to part of the IHHNV genome are found embedded in the genome of penaeids. These were initially described as Type 3A from East Africa, India and Australia, and Type 3B from the western Indo-Pacific region including Madagascar, Mauritius and Tanzania (Tang & Lightner, 2006; Tang *et al*., 2007). Tissues containing the IHHNV-homologous sequences (also known as endogenous viral elements; Taengchaiyaphum *et al*., 2021) in the *P. monodon* genome are not infectious to susceptible host species (Lightner *et al*., 2009; Tang & Lightner, 2006; Tang *et al*., 2007).

2.1.2. Survival and stability in processed or stored samples

IHHNV is believed to be the most stable virus of the known penaeid shrimp viruses. Infected tissues remain infectious after repeated cycles of freeze–thawing and after storage in 50% glycerine (Lightner, 1996; Lightner *et al.,* 1987; Lightner *et al.,* 2009).

2.1.3. Survival and stability outside the host

No data.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IHHNV according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are: yellowleg shrimp (*Penaeus californiensis*), giant tiger prawn

(*Penaeus monodon*), northern white shrimp (*Penaeus setiferus*), blue shrimp (*Penaeus stylirostris*), and white leg shrimp (*Penaeus vannamei*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with IHHNV according to Chapter 1.5 of the *Aquatic Code* are: northern brown shrimp (*Penaeus aztecus*). ~~Evidence is lacking for this species to either confirm that the identity of the pathogenic agent is IHHNV, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection.~~

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: ~~giant river prawn (*Macrobrachium rosenbergii*), northern pink shrimp (*Penaeus duorarum*), western white shrimp (*P. occidentalis*), *kuruma prawn* (*P. japonicus*), green tiger prawn (*P. semisulcatus*), *Hemigrapsus penicillatus,* Argentine stiletto shrimp (*Artemesia longinaris*), Cuata swimcrab (*Callinectes arcuatus),* Mazatlan sole (*Achirus mazatlanus*), yellowfin mojarra (*Gerres cinereus*), tilapias (*Oreochromis* sp.), Pacific piquitinga (*Lile stolifera*) and blackfin snook (*Centropomus medius*).~~

Family	Scientific name	Common name
Achiridae	*Achirus mazatlanus*	Mazatlan sole
Centropomidae	*Centropomus medius*	blackfin snook
Cichlidae	*Oreochromis* sp.	tilapias
Clupeidae	*Lile stolifera*	Pacific piquitinga
Gerreidae	*Gerres cinereus*	yellowfin mojarra
Palaemonidae	*Macrobrachium rosenbergii*	giant river prawn
Penaeidae	*Penaeus duorarum*	northern pink shrimp
	Penaeus occidentalis	western white shrimp
	Penaeus japonicus	*kuruma prawn*
	Penaeus semisulcatus	green tiger prawn
	Artemesia longinaris	Argentine stiletto shrimp
Portunoidea	*Callinectes arcuatus*	Cuata swimcrab
Varunidae	*Hemigrapsus penicillatus*	

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

IHHNV has been detected in all life stages (i.e. eggs, larvae, postlarvae, juveniles and adults) of *P. vannamei.* Nauplii produced from infected broodstock have a high prevalence of infection with IHHNV (Motte *et al.,* 2003).

2.2.4. Distribution of the pathogen in the host

IHHNV targets gills, haematopoietic nodules and haemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, lymphoid organ, parenchymal cells, connective tissue cells and ovaries (Chayaburakul, 2005; Lightner, 1996; Lightner & Redman, 1998).

2.2.5. Aquatic animal reservoirs of infection

Some members of *P. stylirostris* and *P. vannamei* populations that survive IHHNV infection may carry the virus subclinically and infect their progeny or other populations by vertical and horizontal transmission (Bell & Lightner, 1984; Lightner, 1996; Motte *et al*., 2003).

2.2.6. Vectors

IHHNV ~~was found in wild crabs~~ has been detected in many crustacean and non-crustacean species however their ~~(*Hemigrapsus penicillatus*, *Neohelice granulata*), but there were no clinical signs. Adults of *Macrobrachium rosenbergii* are carriers of IHHNV without apparent signs. Although the mussel *Mytilus edulis* is an important reservoir of IHHNV (Wei *et al.,* 2017), its~~ capacity to transmit virus is unknown.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

The effects of infection with IHHNV varies among shrimp species and populations, where infections can be either acute or chronic. For example, in unselected populations of *P. stylirostris*, infection with IHHNV results in acute, usually catastrophic, disease with mortalities approaching 100%. Vertically infected larvae and early postlarvae do not become diseased, but in approximately 35-day-old or older juveniles, gross signs of the disease may be observed, followed by mass mortalities. In horizontally infected juveniles, the incubation period and severity of the disease is somewhat size- or age-dependent, with young juveniles always being the most severely affected. Infected adults seldom show signs of the disease or mortalities (Bell & Lightner, 1984; 1987; Lightner, 1996; Lightner *et al.*, 1983).

In contrast, in populations of *P. vannamei*, some selected lines of *P. stylirostris*, and some populations of *P. monodon*, infection with IHHNV results in a more subtle, chronic disease, runt-deformity syndrome (RDS), in which high mortalities are unusual, but where growth suppression and cuticular deformities are common (Kalagayan *et al*., 1991; Sellars *et al.,* 2019). The severity and prevalence of RDS in infected populations of juvenile or older *P. vannamei* may be related to infection during the larval or early postlarval stages.

~~Infection with IHHNV interferes with normal egg, larval, and postlarval development. When broodstock are used from wild or farmed stocks where the disease is enzootic, hatching success of eggs may be reduced, and survival and culture performance of the larval and postlarval stages lowered (Motte *et al.,* 2003).~~

There was no mortality or clinical signs of disease in *P. vannamei*, *P. monodon* or *P. stylirostris* when experimentally challenged with IHHNV genotypes from Ecuador and Peru (Aranguen Caro *et al.,* 2022). The IHHNV genotypes were found to be within a separate lineage of IHHNV type 2 genotypes circulating within these countries (Aranguen Caro *et al.*, 2022).

In the past, stocks of *P. stylirostris*, juveniles, ~~subadults,~~ and adults showed persistently high mortality rates due to infection with IHHNV. However, selected lines of *P. stylirostris* do not show mortality and appear to be tolerant to this virus. ~~*Penaeus vannamei* and *P. monodon* stocks infected with IHHNV show poor and highly disparate growth and cuticular deformities, particularly bent rostrums and deformed sixth abdominal segments (Jagadeesan *et al.,* 2019; Sellars *et al.,* 2019).~~

In regions where the virus is enzootic in wild stocks, the prevalence of IHHNV has been found in various surveys to range from 0 to 100%. Some reported mean values for IHHNV prevalence in wild stocks are: 26% and 46% in *P. stylirostris* in the lower and upper Gulf of California, respectively (Pantoja *et al.,* 1999); 100% and 57%, respectively, in adult female and adult male *P. stylirostris* from the mid-region of the Gulf of California (Morales-Covarrubias *et al.,* 1999); 28% in wild *P. vannamei* collected from the Pacific coast of Panama (Nunan *et al.,* 2001); from 51 to 63% in *P. vannamei* collected from the Pacific coasts of Ecuador, Colombia and Panama (Motte *et al.,* 2003), and from 6 to 63% in *P. vannamei* broodstock and 49.5% in post-larvae from Mexico (Fernando *et al*., 2016). In farms where IHHNV is present, its prevalence can range from very low to 100%, but high prevalence is typical (Aly *et al*., 2021; Chayaburakul *et al.,* 2004; Lightner, 1996; Lightner *et al.,* 1983).

2.3.2. Clinical signs, including behavioural changes

Animals with this disease may show one or more of these signs, but the pathogen may still be present in the absence of any signs. Clinical signs are non-specific, but juvenile *P. stylirostris* with acute infection with IHHNV show a marked reduction in food consumption, followed by changes in behaviour and appearance. Shrimp of this species infected with IHHNV have been observed to rise slowly in culture tanks to the water surface, where they become motionless and then roll-over and slowly sink (ventral side up) to the tank bottom. Shrimp exhibiting this behaviour may repeat the process for several hours until they become too weak to continue, or until they are attacked and cannibalised by their healthier siblings.

Certain cuticular deformities, specifically a deformed rostrum bent to the left or right, which may be presented by *P. vannamei* and *P. stylirostris* with RDS, are indicative of infection with IHHNV ~~(see Section 2.3.3 *Gross pathology: Infection with IHHNV in* Penaeus vannamei)~~. However, this clinical sign is not always apparent in shrimp populations chronically infected with IHHNV.

In acute disease, *P. stylirostris* may present behavioural changes ~~(see Section 2.3.3 *Gross pathology: Infection with IHHNV in* Penaeus stylirostris)~~ but with RDS, no consistent behavioural changes have been reported for affected shrimp.

Infection with IHHNV interferes with normal egg, larval, and postlarval development. When broodstock are used from wild or farmed stocks where the disease is enzootic, hatching success of eggs may be reduced, and survival and culture performance of the larval and postlarval stages lowered (Motte *et al.,* 2003).

2.3.3. Gross pathology

~~*Infection with IHHNV in* Penaeus stylirostris~~

~~Infection with IHHNV may result in acute disease with very high mortalities in juveniles. Vertically infected larvae and early postlarvae do not become diseased, but in approximately 35-day-old or older juveniles, gross signs of the disease may be observed, followed by mass mortalities. In horizontally infected juveniles, the incubation period and severity of the disease is somewhat size- or age-dependent, with young juveniles always being the most severely affected. Infected adults seldom show signs of the disease or mortalities (Bell & Lightner, 1984; 1987; Lightner, 1996; Lightner *et al.,* 1983). Gross signs are non-specific, but juvenile P. stylirostris with acute infection with IHHNV show a marked reduction in food consumption, followed by changes in behaviour and appearance. Shrimp of this species infected with IHHNV have been observed to rise slowly in culture tanks to the water surface, where they become motionless and then roll over and slowly sink (ventral side up) to the tank bottom. Shrimp exhibiting this behaviour may repeat the process for several hours until they become too weak to continue, or until they are attacked and cannibalised by their healthier siblings. *Penaeus stylirostris* at this stage of infection often have white or buff-coloured spots (which differ in appearance and location from the white spots that sometimes occur in shrimp with WSSV infections) in the cuticular epidermis, especially at the junction of the tergal plates of the abdomen, giving such shrimp a mottled appearance. This mottling later fades in moribund *P. stylirostris* and individuals become more bluish. In *P. stylirostris* and P. monodon with terminal-phase infection with IHHNV, moribund shrimp are often distinctly bluish in colour, with opaque abdominal musculature (Lightner *et al.,* 1983).~~

~~*Infection with IHHNV in* Penaeus vannamei~~

~~RDS, a chronic form of infection with IHHNV, occurs in *P. vannamei.* The severity and prevalence of RDS in infected populations of juvenile or older *P. vannamei* may be related to infection during the larval or early postlarval stages. RDS has also been reported in cultured stocks of *P. stylirostris* and *P. monodon*.~~ Juvenile shrimp with RDS may display a bent (45° to 90° bend to left or right) or otherwise deformed rostrum, a deformed sixth abdominal segment, wrinkled antennal flagella, cuticular roughness, 'bubble-heads', and other cuticular deformities. Populations of juvenile shrimp with RDS display disparate growth with a wide distribution of sizes and many smaller than expected ('runted') shrimp. The coefficient of variation (CV = the standard deviation divided by the mean of different size groups within a population) for populations with RDS is typically greater than 30% and may approach 90%, while populations of juvenile *P. vannamei* and *P. stylirostris* free from infection with IHHNV (and thus RDS-free) usually show CVs of 10–30% (Lightner, 1996; Primavera & Quinitio, 2000).

2.3.4. Modes of transmission and life cycle

Transmission of IHHNV can be by horizontal or vertical. Horizontal transmission has been demonstrated by cannibalism or by contaminated water (Lightner, 1996; Lightner *et al.,* 1983), and vertical transmission via infected eggs (Motte *et al.,* 2003).

2.3.5. Environmental factors

The replication rate of IHHNV at high water temperatures was significantly reduced in a study in which viral replication was compared in *P. vannamei* experimentally infected and held at 24°C and 32°C. After a suitable incubation period, shrimp held at 32°C had approximately 10^2 times lower viral load than shrimp held at 24°C (Montgomery-Brock *et al*., 2007).

2.3.6. Geographical distribution

Infection with IHHNV has been reported from cultured shrimp in most of the major shrimp-culturing regions of the world including Asia, Oceania, North and South America and the Middle East.

IHHNV homologous sequences have been found within the genome of *P. monodon* from East Africa, Australia, and the western Indo-Pacific region (Tang & Lightner, 2006; Tang *et al.*, 2007). These sequences do not represent viral DNA (refer Section 2.1.1 *Aetiological agent*).

See OIE WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy including blocking agents

No scientifically confirmed reports.

2.4.3. Immunostimulation

No scientifically confirmed reports.

2.4.4. Breeding resistant strains

Selected stocks of *P. stylirostris* that are resistant to infection with IHHNV have been developed and these have had some successful application in shrimp farms (Lightner, 1996). However, lines of *P. stylirostris* bred for resistance to infection with IHHNV (Tang *et al.,* 2000) do not have increased resistance to other diseases, such as white spot syndrome virus (WSSV), so their use has been limited. In some stocks a genetic basis for IHHNV susceptibility in *P. vannamei* has been reported (Alcivar-Warren *et al.,* 1997).

2.4.5. Inactivation methods

IHHNV is a stable shrimp virus; infected tissues remain infectious after repeated cycles of freeze–thawing and after storage in 50% glycerine (Lightner, 1996; Lightner et al., 2009).

2.4.6. Disinfection of eggs and larvae

IHHNV is transmitted vertically by the transovarian route (Motte *et al.,* 2003). Disinfection of eggs and larvae is good management practice (Chen *et al.,* 1992) that may reduce IHHNV contamination of spawned eggs and larvae but is not effective for preventing transovarian transmission of IHHNV (Motte *et al.,* 2003).

2.4.7. General husbandry

Some husbandry practices have been successful in preventing the spread of IHHNV. Among these has been the application of PCR pre-screening of wild or pond-reared broodstock or their spawned eggs/nauplii and discarding those that test positive for the virus (Motte *et al.,* 2003), as well as the development of specific pathogen-free (SPF) shrimp stocks of *P. vannamei* and *P. stylirostris* (Lightner, 2005). The latter has proven to be the most successful husbandry practice for the prevention and control of infection with IHHNV (Lightner, 2005).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Infection with IHHNV may be below detection limits in spawned eggs and larval stages, so these life stages are not suitable for surveillance to demonstrate freedom from infection with IHHNV.

3.2. Selection of organs or tissues

IHHNV infects tissues of ectodermal and mesodermal origin. The principal target tissues for IHHNV include connective tissue cells, the gills, haematopoietic nodules and haemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, and lymphoid organ parenchymal cells (Lightner, 1996; Lightner & Redman, 1998). Hence, whole shrimp (e.g. larvae or postlarvae) or tissue samples containing the aforementioned target tissues are suitable for most tests using molecular methods.

3.3. Samples or tissues not suitable for pathogen detection

Enteric tissues (e.g. the hepatopancreas, the midgut or its caeca) are inappropriate samples for detection of IHHNV (Lightner, 1996; Lightner & Redman, 1998). Shrimp eyes contain PCR inhibitors and their use should be avoided.

3.4. Non-lethal sampling

Haemolymph or excised pleopods may be collected and used for testing when non-lethal testing of valuable broodstock is necessary (Lightner, 1996; Lightner & Redman, 1998).

3.5. Preservation of samples for submission

For routine histology or molecular assays, and guidance on preservation of samples for the intended test method see Chapter 2.2.0.

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.2.5 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.2.2 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not relevant.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger shrimp should be processed and tested individually. Small life stages such as PL can be pooled to obtain the minimum amount of material for ~~virus isolation or~~ molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.

Shaded boxes = Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology						++	++	NA		~~++~~	~~++~~	~~NA~~
Cell culture												
Real-time PCR	~~++~~	+++	+++	1	++	+++	+++	1	++	++	++	1
Conventional PCR	~~+~~	++	++	1	++	++	++	1	~~++~~	~~++~~	~~++~~	~~1~~
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation						+	+	1		++	++	1
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods[3]												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); NA = not available; PCR = polymerase chain reaction;
LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

No reliable methods have been developed for direct microscopic pathology.

4.2. Histopathology and cytopathology

Presumptive acute infections in *P. stylirostris* can be readily diagnosed using routine haematoxylin and eosin (H&E) stained sections whereas chronic infection are much more difficult to diagnose using these staining methods. For diagnosis of chronic infections and confirmation of acute infections however, the use of molecular methods is required for IHHNV detection (e.g. by PCR or application of IHHNV-specific DNA probes to dot-blot hybridisation tests or *in-situ* hybridisation [ISH] of histological sections).

Histological demonstration of prominent intranuclear, Cowdry type A inclusion bodies, provides a provisional diagnosis of infection with IHHNV. These characteristic IHHNV inclusion bodies are eosinophilic and often haloed (with H&E stains of tissues preserved with fixatives that contain acetic acid, such as Davidson's AFA and Bouin's solution) (Bell & Lightner, 1988; Lightner, 1996), intranuclear inclusion bodies within chromatin-marginated, hypertrophied nuclei of cells in tissues of ectodermal (epidermis, hypodermal epithelium of fore- and hindgut, nerve cord and nerve ganglia) and mesodermal origin (haematopoietic organs, antennal gland, gonads, lymphoid organ, and connective tissue). Intranuclear inclusion bodies caused by infection with IHHNV may be easily confused with developing intranuclear inclusion bodies caused by WSSV infection. ISH assay (see Section 4.6 In-situ *hybridisation*) of such sections with a DNA probe specific to IHHNV provides a definitive diagnosis of infection with IHHNV (Lightner, 1996a; 2011; Lightner & Redman, 1998a).

The use of Davidson's fixative (containing 33% ethyl alcohol [95%], 22% formalin [approximately 37% formaldehyde], 11.5% glacial acetic acid and 33.5% distilled or tap water) is highly recommended for all routine histological studies of shrimp (Bell & Lightner, 1988; Lightner, 1996a). To obtain the best results, dead shrimp should not be used. Only live, moribund, or compromised shrimp should be selected for fixation and histological examination. Selected shrimp are killed by injection of fixative directly into the hepatopancreas; the cuticle over the cephalothorax and abdomen just lateral to the dorsal midline is opened with fine-pointed surgical scissors to enhance fixative penetration (the abdomen may be removed and discarded), the whole shrimp (or cephalothorax) is immersed in fixative for 24 to 48 hours, and then transferred to 70% ethyl alcohol for storage. After transfer to 70% ethyl alcohol, fixed specimens may be transported by wrapping in cloth or a paper towel saturated with 70% ethyl alcohol and packed in leak-proof plastic bags.

4.3. Cell culture for isolation

IHHNV has not been grown *in vitro*. No crustacean cell lines exist.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

There are multiple ~~geographical~~ variants of IHHNV, some of which are not detected by ~~all of the~~ some available methods. Two primer sets, 392F/R and 389F/R, are the most suitable for detecting all the known genetic variants of IHHNV (Tang *et al*., 2000; 2007). However, these tests also detect non-infectious endogenous viral elements (EVE) within the *P. monodon* genome (previously known as types 3A and 3B), which are inserted into the genome of certain stocks of *P. monodon* from the western Indo-Pacific, East Africa, Australia and India (Saksmerprome *et al.,* 2011; Taengchaiyaphum *et al.,* 2022; Tang & Lightner, 2006; Tang *et al.,* 2007). As these PCR methods may result in positive test results in uninfected *P. monodon,* positive results should be confirmed by a method that detects IHHNV but not the IHHNV-related EVEs.

PCR primers have been developed that can detect the IHHNV sequence but do not amplify IHHNV-related EVEs present in the *P. monodon* stocks from Africa, Australia (Tang *et al.,* 2007), or Thailand (Saksmerprome *et al.,* 2011).

Primer set 309F/R amplifies only a genomic segment of IHHNV types 1 and 2 ~~(the infectious forms of IHHNV)~~, but not the non-infectious EVEs within the *P. monodon* genome (Tang & Lightner, 2006; Tang *et al.,* 2007). ~~Primer set MG831F/R reacts only with non-infectious EVEs within the *P. monodon* genome (Tang *et al.,* 2007).~~ Hence, confirmation of unexpected positive or negative PCR results for IHHNV with a second primer set, or use of another diagnostic method (i.e. histology, bioassay, ISH) is highly recommended.

4.4.1. Real-time PCR

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~

Real-time PCR methods have been developed for the detection of IHHNV (Dhar *et al.,* 2001; Tang & Lightner, 2001). A ~~highly sensitive~~ SYBR Green real-time PCR targeting a segment of the IHHNV genome ~~that is~~ considered less susceptible to endogenisation was developed (Encinas-Garcia *et al.,* 2015). ~~More recently,~~ A TaqMan real-time assay ~~capable of~~ developed to differentiate ~~endogenous virus element~~ EVEs from infectious form of IHHNV in *P. monodon* has been reported (Cowley *et al.,* 2018); however, analysis of a *P. monodon* whole genome sequence has identified 100% primer and probe sequence matches to EVEs (Taengchaiyaphum *et al.,* 2022). The real-time PCR method ~~using TaqMan chemistry~~ described in Table 4.4.1 ~~below for IHHNV~~ generally follows the method used in Tang & Lightner (2001).

Table 4.4.1. Primers and probes for real-time PCR detection of IHHNV

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1* Tang & Lightner, 2001; GenBank Acc. No AF218266			
IHHNV and IHHNV-related EVEs non-structural protein	Fwd IHHNV1608F: TAC-TCC-GGA-CAC-CCA-ACC-A Rev IHHNV1688R: GGC-TCT-GGC-AGC-AAA-GGT-AA Probe: FAM-ACC-AGA-CAT-AGA-GCT-ACA-ATC-CTC-GCC-TAT-TTG-TAMRA	300 nM primers 150 nM probe	40 cycles of: 95°C/1 sec and 60°C/20 sec

***NOTE** – this method will amplify EVEs within the genome of* P. monodon*. Positive results in this species must be confirmed by a method that does not react with IHHNV EVEs.*

~~i) The PCR primers and TaqMan probe are selected from a region of the IHHNV genomic sequence (GenBank AF218266) that encodes for a non structural protein. The upstream (IHHNV1608F) and downstream (IHHNV1688R) primer sequences are: 5'-TAC-TCC-GGA-CAC-CCA-ACC-A-3' and 5'-GGC-TCT GGC AGC AAA GGT AA 3', respectively. The TaqMan probe 5' ACC AGA CAT AGA GCT ACA ATC-CTC-GCC-TAT-TTG-3'), is synthesised and labelled with FAM on the 5' end and TAMRA on the 3' end.~~

~~ii) Preparation of DNA template: DNA extracted from tissues or haemolymph that was preserved in 95% ethanol and then dried. A control consisting of tissues or haemolymph from known negative animals should be included during the DNA extraction step. The DNA can be extracted by a variety of methods. Commercial DNA extraction kits include QIAamp DNA Mini Kit (Qiagen), MagMax™ Nucleic Acid kits (Life Technologies), or Maxwell® 16 Cell LEV DNA Purification Kit (Promega), or DNazol (Life Technologies). Spectrophotometric readings of the final DNA will indicate the purity of the DNA and the amount of total DNA extracted from the sample.~~

~~iii) The real-time PCR reaction mixture contains: TaqMan Fast virus 1-step Master Mix (Life Technologies, or commercially available equivalent reagents), 0.3 µM of each primers, 0.15 µM of TaqMan probe, 5–50 ng DNA, and water in a reaction volume of 20 µl. For optimal results, the reaction mixture should be vortexed and mixed well.~~

~~iv) The cycling profile is: initial denaturation of 20 seconds at 95°C, followed by 40 cycles of denaturation at 95°C for 1 second and annealing/extension at 60°C for 20 seconds.~~

4.4.2. Conventional PCR

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~

Several one-step PCR methods (Krabsetsve *et al.,* 2004; Nunan *at al.,* 2000; Shike *et al.,* 2000; Tang *et al.,* 2000; 2007), and a number of commercial PCR kits are available for IHHNV detection. Nested methods are also available. In addition to IHHNV, some of these methods will amplify EVEs in *Penaeus monodon*. Positive results in *P. monodon* should be followed up with other methods that will not react with EVEs. ~~In the event that results are ambiguous using the 389F/R 'universal' primer set, it is recommended to use primers from a different region of the genome for confirmatory testing. In this case, that would mean using primers 77012F/77353R or the 392F/R primer sets and follow up with sequencing of PCR amplicons for confirmation.~~

Table 4.4.2.1. Recommended primer sets for ~~one-step~~ conventional PCR detection of IHHNV

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1* Tang *et al.*, 2007; GenBank Acc. No. AF218266; 389bp product			
IHHNV and IHHNV-related EVEs Non-structural protein	Fwd 389F: CGG-AAC-ACA-ACC-CGA-CTT-TA Rev 389R: GGC-CAA-GAC-CAA-AAT-ACG-AA	200 nM	35 cycles of: 94°C/30 sec, 60°C/30 sec, and 72°C/30 sec
Method 2* Nunan *et al.*, 2000; GenBank Acc. No AF218266; 356bp product			
IHHNV and IHHNV-related EVEs Between the non-structural and capsid protein-coding regions	Fwd 77012F: TAC-TCC-GGA-CAC-CCA-ACC-A Rev 77353R: GGC-TCT-GGC-AGC-AAA-GGT-AA	1000 nM	35 cycles of: 95°C/30 sec, 60°C/30 sec, and 72°C/30 sec
Method 3* Tang *et al.*, 2000; GenBank Acc. No AF218266; 392bp product			
IHHNV and IHHNV-related EVEs Non-structural protein	Fwd 392F: GGG-CGA-ACC-AGA-ATC-ACT-TA Rev 392R: ATC-CGG-AGG-AAT-CTG-ATG-TG	300 nM	35 cycles of: 95°C/30 sec, 60°C/30 sec, and 72°C/30 sec
Method 4 Tang *et al.*, 2007; GenBank Acc. No AF218266; 309bp product			
IHHNV ORF1	Fwd 309F: TCC-AAC-ACT-TAG-TCA-AAA-CCA-A Rev 309R: TGT-CTG-CTA-CGA-TGA-TTA-TCC-A	200 nM	35 cycles of: 94°C/30 sec, 55°C/30 sec, and 72°C/30 sec

***NOTE** – these methods will amplify EVEs within the genome of* P. monodon*. Positive results in this species must be confirmed by a method that does not react with IHHNV EVEs.*

~~Primer~~	~~Product~~	~~Sequence (5'-3')~~	~~G+C%/Temp.~~	~~GenBank & References~~	~~Specificity~~
~~389F~~	~~389 bp~~	~~CGG-AAC-ACA-ACC-CGA-CTT-TA~~	~~50%/72°C~~	~~AF218266~~	~~All genetic variants of IHHNV~~
~~389R~~		~~GGC-CAA-GAC-CAA-AAT-ACG-AA~~	~~45%/71°C~~	~~(Tang *et al.*, 2007)~~	~~and IHHNV-related EVEs~~
~~77012F~~	~~356 bp~~	~~ATC-GGT-GCA-CTA-CTC-GGA~~	~~50%/68°C~~	~~AF218266~~	~~Not given in the reference~~
~~77353R~~		~~TCG-TAC-TGG-CTG-TTC-ATC~~	~~55%/63°C~~	~~(Nunan *et al.*, 2000)~~	
~~392F~~	~~392 bp~~	~~GGG-CGA-ACC-AGA-ATC-ACT-TA~~	~~50%/68°C~~	~~AF218266~~	~~All genetic variants of IHHNV and IHHNV-related EVEs~~
~~392R~~		~~ATC-CGG-AGG-AAT-CTG-ATG-TG~~	~~50%/71°C~~	~~(Tang *et al.*, 2000)~~	
~~309F~~	~~309 bp~~	~~TCC-AAC-ACT-TAG-TCA-AAA-CCA-A~~	~~36%/68°C~~	~~AF218266~~	~~IHHNV but not IHHNV-related EVEs~~

Primer	Product	Sequence (5′-3′)	G+C%/Temp.	GenBank & References	Specificity
309R		TGT-CTG-CTA-CGA-TGA-TTA-TCC-A	40%/69°C	(Tang *et al.*, 2007)	
MG831F	831 bp	TTG-GGG-ATG-CAG-CAA-TAT-CT	45%/58°C	DQ228358	IHHNV-related EVEs but not IHHNV
MG831R		GTC-CAT-CCA-CTG-ATC-GGA-CT	55%/62°C	(Tang *et al.*, 2007)	

NOTE: Primers 389F/R and 392F/R described above are from the nonstructural protein-coding region of the IHHNV genome. Primers 77012F/77353R are from a region in between the nonstructural and capsid protein-coding region of the genome. In the event that results are ambiguous using the 389F/R 'universal' primer set, it is recommended to use primers from a different region of the genome for confirmatory testing. In this case, that would mean using primers 77012F/77353R or the 392F/R primer sets and follow-up with sequencing of PCR amplicons for confirmation.

General PCR method for IHHNV: the PCR method described below for IHHNV generally follows the methods outlined in Tang *et al.* (2007) and Nunan *et al.* (2000). However, recent minor modifications including the sources of the reagents and the use of an automated DNA extraction instrument are acceptable. The modifications include DNA extraction method, choice of primers (Table 4.4.2.1), and the volume of reaction. These slightly modified methods have been validated in accordance with Chapter 1.1.2 *Principles and methods of validation of diagnostic assays for infectious diseases* and do not affect the diagnostic performance of the assay.

i) Use as a template, the extraction of DNA templates is the same as that described above. Use 1–5 µl of extracted DNA as a template per 25 µl reaction volume.

ii) The following controls should be included in every PCR assay for IHHNV: (a) DNA from a known negative tissue sample; (b) DNA from a known positive sample (either from tissue or haemolymph or from a plasmid clone that contains the fragment that the specific set of primers amplifies; and (c) a 'no template' control.

iii) Use as primers, primers 389F and 389R, which elicit a band 389 bp in size from IHHNV-infected material, or primers 77012F and 77353R, which elicit a band 356 bp in size from IHHNV-infected material. Prepare primers at 10 µM in distilled water.

iv) If PuReTaq™ Ready-To-Go PCR Beads (GE Healthcare) are used, the PCR profile involves a 3–5 minutes at 95°C to denature DNA followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and final extension at 72°C for 5 minutes.

v) Prepare a 'Master Mix' consisting of water and primers.

vi) For a 25 µl reaction mix, add 24 µl Master Mix to each tube and then add 1 µl of the DNA template to be tested.

vii) Vortex each tube, spin quickly to bring down all liquid. Insert tubes into the thermal cycler and start the PCR program.

viii) After PCR, run 6–10 µl of the sample in a 1.5% agarose gel (containing SYBR™ Safe (Thermo Fisher Scientific) or equivalent to stain the DNA). Look for the 389 bp band (if using primers 389F and 389R) or for the 356 bp band (if using primers 77012F and 77353R). Bands are not always seen, as it is necessary to have at least 10 ng DNA μl^{-1} to see DNA in a gel. A direct sequencing of amplified products can be performed through gel extraction of a PCR band with correct size and the sequencing primer(s) used for amplification to confirm the presence of IHHNV.

4.4.3. Other nucleic acid amplification methods

Loop-mediated isothermal amplification (LAMP) assays and a real-time isothermal recombinase polymerase amplification (RPA) assay are available to detect and confirm IHHNV infection have been published (Arunrut *et al.*, 2011; Sun *et al.*, 2006; Xia *et al.*, 2015), however, they are currently not recommended as they are not sufficiently validated.

4.5. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced, analysed and compared with published sequences.

~~PCR products may be directly sequenced or cloned and sequenced when necessary to confirm infection with IHHNV, to identify false positives or nonspecific amplification, or to distinguish the amplified products from the infectious form of the virus and demonstrate the presence of the non-infectious IHHNV-related EVEs in the host genome (Tang & Lighter, 2006).~~

4.6. *In-situ* hybridisation

Direct detection methods using DNA probes specific for IHHNV are available in dot-blot and ISH formats. The ISH method uses a DIG-labelled DNA probe for IHHNV and generally follows the methods outlined in Mari *et al*. (1993) and Lightner (1996).

Gene probe and PCR methods provide greater diagnostic specificity and sensitivity than traditional techniques that employ classic histological approaches. Furthermore, these methods have the added advantage of being applicable to non-lethal testing of valuable broodstock shrimp. A haemolymph sample may be taken with a tuberculin syringe, or an appendage (a pleopod for example) may be biopsied (Bell *et al*., 1990), and used as the sample for a dot-blot hybridisation test.

4.7. Immunohistochemistry

Not relevant.

4.8. Bioassay

If SPF shrimp are available, the following bioassay method is based on Tang *et al.* (2000), is suitable for IHHNV diagnosis.

i) For bioassay, feed the minced shrimp tissue suspected of being infected with IHHNV to the indicator shrimp species (e.g. SPF *P. vannamei* and *P. stylirostris* at the PLs or juvenile stage) at 10% of their body weight twice daily for 1 day~~s~~.

ii) For the following, the indicator shrimp were maintained on a pelletised ration.

iii) Examine moribund shrimp grossly or by using the methods described above. There may be no apparent mortality during the experimental period.

iv) If at 30 days after feeding there are still no moribund shrimp and all test results are negative, then it is safe to conclude that the bioassay results are negative.

Known IHHNV positive and negative control groups should be included in the bioassay.

4.9. Antibody- or antigen-based detection methods

None has been successfully developed.

4.10. Other methods

Not available.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Conventional PCR and/or real-time PCR are the recommended test for surveillance to demonstrate freedom from infection with IHHNV in apparently healthy populations as described in Sections 4.4.1 and 4.4.2.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [8]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. ~~Geographical~~ Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with IHHNV shall be suspected if at least one of the following criteria is met:

i) Positive result by conventional PCR

ii) Positive result by real-time PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with IHHNV is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR and a positive result by conventional PCR ~~targeting non-overlapping regions of the viral genome and~~ followed by amplicon sequencing

ii) ~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with IHHNV shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Histopathology consistent with IHHNV infection

iii) Positive result by conventional PCR

~~iii~~ iv) Positive result by real-time PCR

~~iv) Histopathology consistent with IHHNV infection~~

v) Positive result by *in-situ* hybridisation

[8] For example transboundary commodities.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with IHHNV is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR and a positive result by conventional PCR ~~targeting non-overlapping regions of the viral genome and~~ followed by amplicon sequencing

ii) ~~Histopathology consistent with IHHNV infection coupled with~~ A positive result by *in-situ* hybridisation and ~~detection of IHHNV~~ a positive result by real-time PCR

iii) ~~Histopathology consistent with IHHNV infection coupled with~~ A positive result by *in-situ* hybridisation and ~~detection of IHHNV by~~ a positive result by conventional PCR ~~and~~ followed by amplicon sequencing

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests [under study]

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with IHHNV is provided in Table 6.3.1 (none available). This information can be used for the design of surveys for infection with IHHNV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2 and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
PCR: = polymerase chain reaction.

7. References

ALY S.M., MANSOUR S.M., THABET R.Y. & MABROK M. (2021). Studies on infectious myonecrosis virus (IMNV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) in cultured penaeid shrimp in Egypt. *Dis. Aquat. Org.*, **143**, 57–67.

ARANGUREN CARO L.F., GOMEZ-SANCHEZ M.M., PIEDRAHITA Y., MAI H.N., CRUZ-FLORES R., ALENTON R.R.R.& DHAR A.K. (2022). Current status of infection with infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the Peruvian and Ecuadorian shrimp industry. *PLoS One*, 17(8):e0272456. doi: 10.1371/journal.pone.0272456.

ARUNRUT N., PROMBUN P., SAKSMERPROME V., FLEGEL T. W. & KIATPATHOMCHAI W. (2011). Rapid and sensitive detection of infectious hypodermal and hematopoietic necrosis virus by loop-mediated isothermal amplification combined with a lateral flow dipstick. *J. Virol. Methods,* **171**, 21–25.

BELL T.A. & LIGHTNER D.V. (1984). IHHN virus: infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. *Aquaculture*, **38**, 185–194.

BELL T.A. & LIGHTNER D.V. (1987). IHHN disease of *Penaeus stylirostris*: effects of shrimp size on disease expression. *J. Fish Dis*., **10**, 165–170.

BELL T.A. & LIGHTNER D.V. (1988). A Handbook of Normal Shrimp Histology. Special Publication No. 1, World Aquaculture Society, Baton Rouge, Louisiana, USA, 114 pp.

BONAMI J.R., TRUMPER B., MARI J., BREHELIN M. & LIGHTNER D.V. (1990). Purification and characterization of IHHN virus of penaeid shrimps. *J. Gen. Virol*., **71**, 2657–2664.

CHAYABURAKUL K., LIGHTNER D.V., SRIURAIRATTANA S., NELSON K.T. & WITHYACHUMNARNKUL B. (2005). Different responses to infectious hypodermal and hematopoietic necrosis virus (IHHNV) in *Penaeus monodon* and *P. vannamei*. *Dis. Aquat. Org*., **67**, 191–200.

CHAYABURAKUL K., NASH G., PRATANPIPAT P., SRIURARAIRATANA S. & WITHYACHUMNARNKUL B. (2004). Multiple pathogens found in growth-retarded black tiger shrimp *Penaeus monodon* cultivated in Thailand. *Dis. Aquat. Org*., **60**, 89–96.

CHEN S.N., CHANG P.S. & KOU G.H. (1992). Infection route and eradication of *Penaeus monodon* baculovirus (MBV) in larval giant tiger prawns, *Penaeus monodon. In:* Diseases of Cultured Penaeid Shrimp in Asia and the United States, Fulks W. & Main K.L., eds. Oceanic Institute, Honolulu, Hawaii, USA, 177–184.

COWLEY J.A., RAO M., COMAN G.J. & COWLEY J. (2018). Real-time PCR tests to specifically detect Infectious hypodermal and haemopoietic necrosis virus (IHHNV) lineages and an IHHNV endogenous viral element (EVE) integrated in the genome of Black Tiger shrimp (*Penaeus monodon*). *Dis. Aquat. Org*., **129**, 145–158.

DHAR A.K., ROUX M.M. & KLIMPEL K.R. (2001). Detection and quantification of Infectious hypodermal and hematopoeitic necrosis virus and White spot virus in shrimp using real-time quantitative PCR and SYBR green chemistry. *J. Clin. Microbiol*., **39**, 2835–2845.

ENCINAS-GARCIA T., MENDOZA-CANO F., ENRÍQUEZ-ESPINOZA T., LUKEN-VEGA L., VICHIDO-CHÁVEZ R. & SÁNCHEZ-PAZ A. (2015). An improved validated SYBR green-based real-time quantitative PCR assay for the detection of the *Penaeus stylirostris* densovirus in penaeid shrimp. *J. Virol. Methods*, **212**, 53–58.

FERNANDO M.C., ENRIQUEZ-ESPINOZA T., VALENZUELA-CASTILLO A., ENCINAS-GARCIA T. & SANCHEZ-PAZ A. (2016). High Occurrence of the Decapod Penstyldensovirus (PstDV1) Detected in Postlarvae of *Penaeus vannamei* Produced in Commercial Hatcheries of Mexico. *EcoHealth.* **13**, 591–596.

~~JAGADEESAN V., EZHIL PRAVEENA P., OTTA S.K. & JITHENDRAN K.P. (2019). Classical runt deformity syndrome cases in farmed *Penaeus vannamei* along the east coast of India. *In:* BRAQCON 2019: World Brackishwater Aquaculture Conference, Jithendran K.P., Saraswathy R., Balasubramanian C.P., Kumaraguru Vasagam K.P., Jayasankar V., Raghavan R., Alavandi S.V. & Vijayan K.K., eds. Journal of Coastal Research, Special Issue No. 86, pp. 107–111. Coconut Creek (Florida), ISSN 0749-0208.~~

KALAGAYAN G., GODIN D., KANNA R., HAGINO G., SWEENEY J., WYBAN J. & BROCK J. (1991). IHHN virus as an etiological factor in runt-deformity syndrome of juvenile *Penaeus vannamei* cultured in Hawaii. *J. World Aquacult. Soc*., **22**, 235–243.

KRABSETSVE K., CULLEN B.R. & OWENS L. (2004). Rediscovery of the Australian strain of infectious hypodermal and haematopoietic necrosis virus. *Dis. Aquat. Org*., **61**, 153–158.

LIGHTNER D.V. (ED.) (1996). A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA, 304 pp.

LIGHTNER D.V. (2005). Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *J. World Aquaculture Soc.* **36**, 229–248.

LIGHTNER D.V., MOHNEY L.L., WILLIAMS R.R. & REDMAN R.M. (1987). Glycerol tolerance of IHHN virus of penaeid shrimp. *J. World Aquac. Soc*., **18**, 196–197.

LIGHTNER D.V., REDMAN R.M., ARCE S. & MOSS S.M. (2009). Specific pathogen-free (SPF) shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans. In: Shellfish Safety and Quality, Shumway S. & Rodrick G., eds. Woodhead Publishers, London, UK, 384–424.

LIGHTNER D.V. & REDMAN R.M. (1998). Shrimp diseases and current diagnostic methods. *Aquaculture*, **164**, 201–220.

LIGHTNER D.V., REDMAN R.M. & BELL T.A. (1983). Infectious hypodermal and hematopoietic necrosis a newly recognized virus disease of penaeid shrimp. *J. Invertebr. Pathol*., **42**, 62–70.

MARI J., BONAMI J.R. & LIGHTNER D.V. (1993). Partial cloning of the genome of infectious hypodermal and hematopoietic necrosis virus, an unusual parvovirus pathogenic for penaeid shrimps; diagnosis of the disease using a specific probe. *J. Gen. Virol.,* **74**, 2637–2643.

MONTGOMERY-BROCK D., TACON A.G.J., POULOS B., & LIGHTNER D.V. (2007). Reduced replication of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in *Litopenaeus vannamei* held in warm water. *Aquaculture*, **265**, 41–48.

MORALES-COVARRUBIAS M.S., NUNAN L.M., LIGHTNER D.V., MOTA-URBINA J.C., GARZA-AGUIRRE M.C. & CHAVEZ-SANCHEZ M.C. (1999). Prevalence of IHHNV in wild broodstock of *Penaeus stylirostris* from the upper Gulf of California, Mexico*. J. Aquat. Anim. Health*, **11**, 296–301.

MOTTE, E., YUGCHA E., LUZARDO J., CASTRO F., LECLERCQ G., RODRÍGUEZ J., MIRANDA P., BORJA O., SERRANO J., TERREROS M., MONTALVO K., NARVÁEZ A., TENORIO N., CEDEÑO V., MIALHE E. & BOULO V. (2003). Prevention of IHHNV vertical transmission in the white shrimp *Litopenaeus vannamei. Aquaculture,* **219**, 57–70.

NUNAN L.M., POULOS B.T. & LIGHTNER D.V. (2000). Use of polymerase chain reaction (PCR) for the detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in penaeid shrimp. *Mar. Biotechnol*., **2**, 319–328.

NUNAN L.M., ARCE S.M., STAHA R.J. & LIGHTNER D.V. (2001). Prevalence of Infectious hypodermal and hematopoietic necrosis virus (IHHNV) and White spot syndrome virus (WSSV) in *Litopenaeus vannamei* in the Pacific Ocean off the coast of Panama. *J. World Aquacult. Soc*., **32**, 330–334.

PANTOJA C.R., LIGHTNER D.V. & HOLTSCHMIT K.H. (1999). Prevalence and geographic distribution of IHHN parvovirus in wild penaeid shrimp (Crustacea: Decapoda) from the Gulf of California, Mexico*. J. Aquat. Anim. Health*, **11**, 23–34.

PENZES J.J., SODERLUND-VENERMO M., CANUTI M., EIS-HUBINGER A.M., HUGHES J., COTMORE S.F. & HARRACH B. (2020). Reorganizing the family *Parvoviridae*: a revised taxonomy independent of the canonical approach based on host association. *Arch. Virol*., **165**, 2133–2146. https://doi.org/10.1007/s00705-020-04632-4

PRIMAVERA, J.H. & QUINITIO E.T. (2000). Runt-deformity syndrome in cultured giant tiger prawn *Penaeus monodon. J. Crust. Biol*., **20**, 796–802.

SAKSMERPROME V., JITRAKORN S., CHAYABURAKUL K., LAIPHROM S., BOONSUA K. & FLEGEL T.W. (2011). Additional random, single to multiple genome fragments of *Penaeus stylirostris* densovirus in the giant tiger shrimp genome have implications for viral disease diagnosis. *Virus Res*., **160**, 180–190.

SELLARS M.J., COWLEY J.A., MUSSON D., RAO M., MENZIES M.L, COMAN G. J. & MURPHY B.S. (2019). Reduced growth performance of Black Tiger shrimp (*Penaeus monodon*) infected with infectious hypodermal and hematopoietic necrosis virus. *Aquaculture*, **499**, 160–166.

SHIKE H., DHAR A.K., BURNS J.C., SHIMIZU C., JOUSSET F.X., KLIMPEL K.R. & BERGOIN M. (2000). Infectious hypodermal and hematopoietic necrosis virus of shrimp is related to mosquito Brevidensoviruses. *Virology*, **277**, 167–177.

SUN Z.F., HU C. Q., REN C. H. & SHEN Q. (2006). Sensitive and rapid detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in shrimps by loop-mediated isothermal amplification. *J. Virol. Methods,* **131**, 41–46.

TAENGCHAIYAPHUM S., BUATHONGKAM P., SUKTHAWORN S., WONGKHALUANG P., SRITUNYALUCKSANA K. & FLEGEL T.W. (2021). Shrimp Parvovirus Circular DNA Fragments Arise From Both Endogenous Viral Elements and the Infecting Virus. *Front. Immunol*., **12**, 729528. doi: 10.3389/fimmu.2021.729528.

TAENGCHAIYAPHUM S., WONGKHALUANG P., SITTIKANKAEW K., KAROONUTHAISIRI N., FLEGEL T.W. & SRITUNYALUCKSANA K. (2022). Shrimp genome sequence contains independent clusters of ancient and current Endogenous Viral Elements (EVE) of the parvovirus IHHNV. *BMC Genomics*, **23**, 565. doi: 10.1186/s12864-022-08802-3.

TANG K.F.J., DURAND S.V., WHITE B.L., REDMAN R.M., PANTOJA C.R. & LIGHTNER D.V. (2000). Postlarvae and juveniles of a selected line of *Penaeus stylirostris* are resistant to infectious hypodermal and hematopoietic necrosis virus infection. *Aquaculture*, **190**, 203–210.

TANG K.F.J. & LIGHTNER D.V. (2001). Detection and quantification of infectious hypodermal and hematopoietic necrosis virus in penaeid shrimp by real-time PCR. *Dis. Aquat. Org.,* **44**, 79–85.

TANG K.F.J. & LIGHTNER D.V. (2006). Infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the genome of the black tiger prawn *Penaeus monodon* from Africa and Australia. *Virus Res.,* **118**, 185–191.

TANG K.F.J., NAVARRO S.A. & LIGHTNER D.V. (2007). A PCR assay for discriminating between infectious hypodermal and hematopoietic necrosis virus (IHHNV) and the virus-related sequences in the genome of *Penaeus monodon*. *Dis. Aquat. Org.,* **74**, 165–170.

TANG K.F.J., POULOS B.T., WANG J., REDMAN R.M., SHIH H.H. & LIGHTNER D.V. (2003). Geographic variations among infectious hypodermal and hematopoietic necrosis virus (IHHNV) isolates and characteristics of their infection. *Dis. Aquat. Org*., **53**, 91–99.

~~WEI Y.W., FAN D.D. & CHEN J. (2017). The mussel *Mytilus edulis* L. as an important reservoir of infectious hypodermal and hematopoietic necrosis virus (IHHNV). *Aquaculture Res.,* **48**, 1346–1350.~~

XIA X.X., YU Y.X., HU L.H., MANFRED W. & PAN Y.J. (2015). Rapid detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) by real-time, isothermal recombinase polymerase amplification assay. *Arch. Virol.,* **160**, 987–994.

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* *

NB: There are OIE Reference Laboratories for infection with infectious hypodermal and haematopoietic necrosis virus (please consult the OIE web site for the most up-to-date list: http://www.woah.org/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with infectious hypodermal and haematopoietic necrosis virus

NB: FIRST ADOPTED IN 1995 AS INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS;
MOST RECENT UPDATES ADOPTED IN 2018.

CHAPTER 2.2.5.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

1. Scope

Infection with infectious myonecrosis virus means infection with the pathogenic agent infectious myonecrosis virus (IMNV) that is tentatively assigned to the Family *Totiviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Phylogenetic analysis of its RNA-dependent RNA polymerase (RdRp) gene coding sequence indicates that IMNV is most closely related to *Giardia lamblia virus*, a member of the family *Totiviridae* (Fauquet *et al.,* 2005; Lightner, 2011; Nibert, 2007; Poulos *et al.,* 2006).

IMNV particles are icosahedral in shape and 40 nm in diameter, with a buoyant density of 1.366 g ml^{-1} in caesium chloride. The genome consists of a single, double-stranded (ds) RNA molecule of 8226–8230 bp (Loy *et al*., 2015; Naim *et al.,* 2015). Sequencing of the viral genome reveals two non-overlapping open reading frames (ORFs). The first ORF (ORF1, 470–5596 nt) encodes a putative RNA-binding protein and a capsid protein. The coding region of the RNA-binding protein is located in the first half of ORF1 and contains a dsRNA-binding motif in the first 60 amino acids. The second half of ORF1 encodes a capsid protein, as determined by amino acid sequencing, with a molecular mass of 106 kDa. The second ORF (ORF2, 5884–8133 nt) encodes a putative RdRp (Poulos *et al.,* 2006). The most variable region of IMNV genome is located in the first half of ORF1, coinciding with a region which probably encodes the capsid protrusions (Dantas *et al*., 2015).

The complete genomes of IMNV types originating from Brazil and Indonesia have been sequenced and found to be 99.6% identical at the nucleotide level (Poulos *et al.,* 2006; Senapin *et al.,* 2007). The 99.6% full genome sequence identity (and anecdotal information on the introduction of *Penaeus vannamei* stocks from Brazil) indicate that the disease was introduced from Brazil to Indonesia in 2006. A new genotype was analysed in infected samples in 2018 in Indonesia, including an isolate that contains a deletion of 622 amino acids (Mai *et al.,* 2019).

2.1.2. Survival and stability in processed or stored samples

No data.

2.1.3. Survival and stability outside the host

No information available.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IMNV according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are: brown tiger prawn (*Penaeus esculentus*), banana prawn (*P. merguiensis*), and whiteleg shrimp (*P. vannamei*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with IMNV according to Chapter 1.5 of the *Aquatic Code* are: giant tiger prawn (*Penaeus monodon*) and blue shrimp (*P. stylirostris*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: southern brown shrimp (*P. subtilis*).

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Juveniles and subadults of *P. vannamei*, farmed in marine, brackish, and low salinity brackish water, appear to be most severely affected by infection with IMNV (Lightner, 2011; Lightner *et al.,* 2004; Nunes *et al.,* 2004; Poulos *et al.,* 2006).

2.2.4. Distribution of the pathogen in the host

The principal target tissues for IMNV include the striated muscles (skeletal and less often cardiac), connective tissues, haemocytes, and the lymphoid organ parenchymal cells (Lightner, 2011; Lightner *et al.,* 2004; Poulos *et al.,* 2006; Tang *et al.,* 2005).

2.2.5. Aquatic animal reservoirs of infection

Some members of populations of *P. vannamei* that survive IMNV infections or epizootics may carry the virus.

2.2.6. Vectors

Experimental studies have demonstrated that brine shrimp *Artemia franciscana* can act as a vector for IMNV (da Silva *et al*., 2015).

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

In early juvenile, juvenile, or adult *P. vannamei* in regions where infection with IMNV is enzootic, outbreaks of IMNV infections associated with sudden high morbidity and mortality may follow 'stress' events such as capture by cast-netting, feeding and sudden changes in water salinity or temperature (Lightner, 2011; Lightner *et al.,* 2004; Nunes *et al.,* 2004; Poulos *et al.,* 2006). Feed conversion ratios of affected populations can increase from a normal value of ~ 1.5 up to 4.0 or higher (Andrade *et al.,* 2007). Mortalities from infection with IMNV can range from 40% to 70% in cultivated *P. vannamei*.

In regions where infection with IMNV is enzootic in farmed stocks of *P. vannamei*, its prevalence may reach 100% (Andrade *et al.,* 2007; Nunes *et al.,* 2004).

2.3.2. Clinical signs, including behavioural changes

Affected shrimp present with visibly white tails. Such severely affected shrimp may have been feeding just before the onset of stress and may have a full gut. High mortality can occur suddenly and continue for several days. Clinical signs may have a sudden onset following stress events (e.g. capture by cast-netting, feeding, and sudden changes in temperature or salinity).

Only shrimp in the acute phase of disease present behavioural changes. Typically, severely affected shrimp become lethargic during or soon after stress events such as capture by cast-netting, feeding, sudden changes in water temperature, sudden reductions in water salinity, etc.

2.3.3 Gross pathology

Shrimp in the acute phase of disease present focal-to-extensive white necrotic areas in striated (skeletal) muscles, especially in the distal abdominal segments and tail fan, which can become necrotic and reddened in some individual shrimp.

Exposing the paired lymphoid organs (LO) by simple dissection will show that they are hypertrophied (3–4 times their normal size) (Lightner *et al.,* 2004; Poulos *et al.,* 2006).

2.3.4. Modes of transmission and life cycle

IMNV has been demonstrated to be transmitted horizontally by cannibalism (Lightner, 2011; Poulos *et al.,* 2006). Transmission via water probably occurs. Although vertical transmission is suspected from anecdotal evidence, it is not known whether this occurs via transovarial mechanism or by surface contamination of newly spawned eggs.

2.3.5. Environmental factors

Temperature and salinity effects are likely predisposing factors to disease outbreaks, but no experimental data are available (Nunes *et al.,* 2004).

2.3.6. Geographical distribution

Infection with IMNV has been reported to occur in some countries in the Americas, Asia and Africa (Aly *et al.,* 2021; Andrade *et al.,* 2007; Lightner *et al.,* 2004; Naim *et al.,* 2014; Nunes *et al.,* 2004; Poulos *et al.,* 2006; Sahul *et al*., 2017).

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No effective vaccines for infection with IMNV are available.

2.4.2. Chemotherapy including blocking agents

Ctn[15-34], a cathelicidin-derived eicosapeptide was found to demonstrate antiviral activity against IMNV in primary haemocyte cultures (Vieira-Girao *et al.,* 2017).

2.4.3. Immunostimulation

No data.

2.4.4. Breeding resistant strains

There are anecdotal reports of some selected lines of *P. vannamei* having better survival and culture performance in farms where infection with IMNV is enzootic. During a 20-day controlled laboratory study in which the shrimp were challenged with IMNV, some domesticated lines of *P. vannamei* were found to survive better than other lines (White-Noble *et al.,* 2010).

Penaeus monodon and *P. stylirostris*, for which there is incomplete evidence of susceptibility (see section 2.2.2), are considered to be more resistant to infection with IMNV than *P. vannamei* (Tang *et al.,* 2005).

2.4.5. Inactivation methods

No data.

2.4.6. Disinfection of eggs and larvae

While IMNV is believed to be transmitted vertically, there are no scientific data confirming this route of transmission. Disinfection of eggs and larvae (Chen *et al.,* 1992) is a good management practice recommended to reduce the potential for transmission of a number of penaeid shrimp diseases from female spawners to their

eggs or larvae, and the practice may reduce IMNV contamination of spawned eggs and larvae produced from them.

2.4.7. General husbandry

Management practices in endemic areas principally involves exclusion of IMNV from shrimp farms. Broodstock or their spawned eggs or nauplii are PCR-tested and those that test positive are discarded (Andrade *et al.,* 2007). Fallowing and restocking of affected farms or entire culture regions with IMNV-free stocks of *P. vannamei* most suited to local culture conditions has proven to be the most successful for preventing and controlling other virus diseases of shrimp, and should be applicable to control and prevent infection with IMNV (Lee & O'Bryen, 2003; Lightner, 2005; Lightner *et al.,* 2009; Moss & Moss, 2009).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Specimens suitable for testing for infection with IMNV using molecular methods (e.g. RT-PCR, nested RT-PCR, real-time RT-PCR, etc.) include postlarvae (PL), juveniles, subadults and adults. While IMNV may infect all life stages, infection severity, and hence virus load, may be below detection limits in spawned eggs and in larval stages, so these life stages may not be suitable for demonstrating freedom from infection with IMNV unless validated for those life stages.

3.2. Selection of organs or tissues

IMNV infects tissues of mesodermal origin. The principal target tissues in the acute phase of infection with IMNV are the striated muscles (skeletal and less commonly cardiac muscle), connective tissues, haemocytes, and the lymphoid organ tubule parenchymal cells. In chronic infections, the lymphoid organ may be the principal target tissue.

3.3. Samples or tissues not suitable for pathogen detection

IMNV replicates systemically but does not replicate in enteric tissues (e.g. the hepatopancreas, the midgut, or its caeca). Hence, enteric tissues are inappropriate samples for detection of IMNV infection.

3.4. Non-lethal sampling

Haemolymph or excised pleopods may be collected and used when non-lethal testing of valuable broodstock is necessary.

3.5. Preservation of samples for submission

Several factors can affect specimen quality during collection, handling and storage, such as exposure to light, heat, desiccation, and incomplete preservation. Hence, standard operating protocols or recommended practices should be followed at all steps of the diagnostic process.

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0.

3.5.1. Samples for pathogen isolation

Not applicable.

3.5.2. Preservation of samples for molecular detection

Tissue samples (pleopods, cephalothorax, muscle, haemolymph) for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.2.2 of Chapter 2.3.0 *General information* (diseases of fish).

3.5.4. Samples for other tests

Not applicable.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger shrimp should be processed and tested individually. Small life stages such as PL or fry can be pooled to obtain the minimum amount of material for molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts					+	+	+	1				
Histopathology					++	++	++	2				
Cell culture												
Real-time RT-PCR	+	++	++	1	++	++	++	2	++	++	++	2
Conventional RT-PCR	+	++	++	1	++	++	++	1				
Conventional RT-PCR followed by amplicon sequencing									+++	+++	+++	1
***In-situ* hybridisation**					+	+	+	1	+	++	++	1
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods												
Other methods												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test. [give definitions of abbreviations as appropriate; nPCR = nested PCR, etc. NB "RT-PCR" is reserved for reverse-transcription polymerase chain reaction methods. "real-time PCR" should always be stated in full and refers to probe-based and SYBR green assays]
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Stained or unstained tissue squashes of affected skeletal muscle or of the LO may show abnormalities. Tissue squashes of skeletal muscle when examined with phase or reduced light microscopy may show loss of the normal striations. Fragmentation of muscle fibres may also be apparent. Squashes of the LO may show the presence of significant accumulations of spherical masses of cells called lymphoid organ spheroids (LOS) amongst normal LO tubules.

4.2. Histopathology and cytopathology

Infection with IMNV in the acute and chronic phases can be presumptively diagnosed using histology (Bell & Lightner, 1988; Lightner, 2011; Lightner *et al.,* 2004; Poulos *et al.,* 2006). However, the lesions in striated muscles and LO are not pathognomonic for infection with IMNV. White tail disease of penaeid shrimp caused by the *P. vannamei* nodavirus (PvNV) can mimic infection with IMNV (Tang *et al.,* 2007).

Haematoxylin and eosin stained tissue sections from shrimp with acute-phase infection with IMNV present myonecrosis with characteristic coagulative necrosis of striated (skeletal) muscle fibres, often with marked oedema among affected muscle fibres. Some shrimp may present a mix of acute and older lesions. The affected muscle fibres appear to progress from presenting coagulative necrosis to liquefactive necrosis, which is accompanied by moderate infiltration and accumulation of haemocytes. In the most advanced lesions, haemocytes and inflamed muscle fibres are replaced by a loose matrix of fibrocytes and connective tissue fibres that are interspersed with haemocytes and foci of (presumed) regenerating muscle fibres (Lightner *et al.,* 2004; Poulos *et al.,* 2006).

Significant hypertrophy of the LO caused by accumulations of LOS is a highly consistent lesion in shrimp with acute or chronic-phase infection with IMNV lesions. Often, many ectopic LOS are found in other tissues not near the main body of the LO. Common locations for ectopic LOS include the haemocoelom in the gills, heart, near the antennal gland tubules, and ventral nerve cord (Lightner *et al.,* 2004; Poulos *et al.,* 2006).

4.3. Cell culture for isolation

No crustacean cell lines exist, but IMNV was observed to propagate in C6/36 subclone of *Aedes albopictus* cell line (Kumar *et al.,* 2020). Performance of the test should be confirmed before being recommended.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

Published methods are available for the molecular detection of IMNV by *in-situ* hybridisation (ISH), nested RT-PCR and quantitative real-time RT-PCR (Andrade *et al.,* 2007; Poulos *et al.,* 2006; Tang *et al.,* 2005). A nested RT-PCR kit for detection of the virus is available commercially.

4.4.1. Real-time RT-PCR

A real-time RT-PCR method was developed to detect and quantify IMNV in shrimp tissue. The method which can detect as few as 10 IMNV RNA copies μl^{-1} total RNA (Andrade *et al.,* 2007) is summarised below.

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Andrade *et al.,* 2007; GenBank Accession No. AY570982			

IMNV Capsid protein gene	Fwd IMNV412F: GGA-CCT-ATC-ATA-CAT-AGC-GTT-GCA Rev IMNV545R: AAC-CCA-TAT-CTA-TTG-TCG-CTG-GAT Probe: CCA-CCT-TTA-CTT-TCA-ATA-CTA-CAT-CAT-CCC-CGG	300 Nm 200 nM	40 cycles of: 95°C/3 sec and 60°C/30 sec

4.4.2. Conventional PCR

The nested RT-PCR method to detect IMNV uses two PCR primer sets that produce a 328 bp one-step amplicon and139 bp two-step amplicon. The 1-step PCR can detect as little as 100 IMNV RNA copies and the 2-step PCR can detect in the order of 10 IMNV RNA copies (Poulos & Lightner, 2006).

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Poulos & Lightner, 2006; GenBank : KJ636783.2; 328/139 bp			
IMNV Capsid protein gene (nested-PCR)	Outer Fwd 4587F: CGA-CGC-TGC-TAA-CCA-TAC-AA Rev 4914R: ACT-CGG-CTG-TTC-GAT-CAA-GT Inner Fwd 4725 NF: GGC-ACA-TGC-TCA-GAG-ACA Rev 4863 NR: AGC-GCT-GAG-TCC-AGT-CTT-G	200 nM 620 nM	45 cycles of: 95°C/45 sec; 60°C/45 sec; 60°C/7 min 39 cycles of: 95°C/30 sec, 65°C/30 sec, 72°C/30 sec; 72°C/2 min

4.4.3. Other nucleic acid amplification methods

None.

4.5. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced and analysed and compared with published sequences.

4.6. *In-situ* hybridisation

DNA probe for ISH detection of IMNV

A cDNA library was generated from RNA extracted from purified IMNV. A IMNV-specific ISH DNA probe is prepared from clone IMNV-317 by PCR labelling with digoxigenin-11-dUTP (DIG). The PCR primers used for amplification of the 993 bp probe are IMNV993F (5'-AAC-ACA-AAA-TCT-GCC-AGC-AA-3') and IMNV993R (5'-CCC-AAC-CAC-CCA-AAT-TCA-TA-3'). Following PCR, the DIG-labelled DNA probe is precipitated with ethanol, re-suspended in water and stored at –20°C until used. The ISH procedure for detecting IMNV follows that outlined by Tang *et al.* (2005). Negative and positive controls should be sourced from PCR-confirmed uninfected and infected shrimp, respectively.

4.7. Immunohistochemistry

Monoclonal antibodies have been generated using recombinant IMNV capsid protein fragments to immunise mice (Kunanopparat et al., 2011). Immunohistochemical analysis demonstrated strong reactivity in muscle, gill, heart, LO and connective tissue derived from IMNV-infected *P. vannamei* similar to that demonstrated by *in-situ* hybidisation (Tang *et al*., 2005). There was no cross-reactivity to tissues derived from uninfected shrimp or shrimp infected with other viral pathogens such as WSSV, YHV, TSV among others.

4.8. Bioassay

Not applicable.

4.9. Antibody- or antigen-based detection methods

None are recommended, however an immunochromatographic strip test has been developed (Chaivisuthangkura *et al*., 2013) using the monoclonal antibodies developed by Kunanopparat *et al*. (2011). While the test is simple, fast and low-cost it is approximately 300-fold less sensitive than one-step RT-PCR (Chaivisuthangkura *et al*., 2013).

4.10. Other methods

A chromatographic method for detection of PCR amplicons has been developed (Koiwai *et al.,* 2018).

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time RT-PCR is the recommended test for surveillance to demonstrate freedom of infection with IMNV in apparently healthy populations as described in Section 4.1.1.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [9]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with IMNV shall be suspected if at least one of the following criteria is met:

i) Histopathology consistent with the presence of the pathogen or the disease

ii) Positive result by real-time RT-PCR

iii) Positive result by conventional RT-PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with IMNV is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR and positive result by conventional RT-PCR followed by amplicon sequencing

ii) Histopathology consistent with IMNV infection coupled with *in-situ* hybridisation and detection of IMNV in a tissue sample by real-time RT-PCR

iii) Histopathology consistent with IMNV infection coupled with *in-situ* hybridisation and detection of IMNV in a tissue sample by conventional RT-PCR followed by amplicon sequencing

[9] For example transboundary commodities.

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with IMNV shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Positive result by conventional RT-PCR

iii) Positive result by real-time RT-PCR

iii) Histopathology consistent with the presence of the pathogen or the disease

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with IMNV is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR and a positive result by conventional RT-PCR followed by amplicon sequencing

ii) Positive result by *in-situ* hybridisation and a positive result by real-time RT-PCR

iii) Positive result by *in-situ* hybridisation and a positive result by conventional RT-PCR followed by amplicon sequencing

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with IMNV are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with IMNV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Experimentally infected SPF *P. vannamei*	abdominal muscle	*P. vannamei*	100 (30)	100 (30)	Histopathology	Andrade *et al.* (2007)

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study, PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR								

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study, PCR: = polymerase chain reaction.

7. References

ALY S.M., MANSOUR S.M., THABET R.Y. & MABROK M. (2021). Studies on infectious myonecrosis virus (IMNV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) in cultured penaeid shrimp in Egypt. *Dis. Aquat. Org.,* **143**, 57–67. doi: 10.3354/dao03556. PMID: 33570040.

ANDRADE T.P.D., SRISUVAN T., TANG K.F.J. & LIGHTNER D.V. (2007). Real-time reverse transcription polymerase chain reaction assay using TaqMan probe for detection and quantification of infectious myonecrosis virus (IMNV). *Aquaculture*, **264**, 9–15.

BELL T.A. & LIGHTNER D.V. (1988). A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society, Baton Rouge, LA, USA, 114 p.

CHAIVISUTHANGKURA P., SENAPIN S., WANGMAN P., LONGYANT S. & SITHIGORNUL P. (2013). Simple and rapid detection of infectious myonecrosis virus using an immunochromatographic strip test. *Arch Virol*., **158**, 1925–1930.

CHEN S.N., CHANG P.S. & KOU G.H. (1992). Infection route and eradication of *Penaeus monodon* baculovirus (MBV) in larval giant tiger prawns, *Penaeus monodon. In:* Diseases of Cultured Penaeid Shrimp in Asia and the United States, Fulks W. & Main K.L., eds. Oceanic Institute, Honolulu, Hawaii, USA, 177–184.

DANTAS M.D., CHAVANTE S.F., TEIXEIRA D.I., LIMA J.P. & LANZA D.C. (2015). Analysis of new isolates reveals new genome organization and a hypervariable region in infectious myonecrosis virus (IMNV). *Virus Res*., **20**, 66–71. doi: 10.1016/j.virusres.2015.03.015. Epub 2015 Apr 4. PMID: 25849112.

DA SILVA S.M.B.C., LAVANDER H.D., DE SANATANA LUNA M.M., DA SILVA A.O.M.E., GALVEZ A.O. & COIMBRA M.R.M. (2015). *Artemia franciscana* as a vector for infectious myonecrosis virus (IMNV) to *Litopenaeus vannanmei* juvenile. *J. Invertebr. Pathol*., **126,** 1–5.

FAUQUET C.M., MAYO M.A., MANILOFF J., DESSELBERGER U. & BALL L.A., EDITORS (2005). Totiviridae. *In:* Virus Taxonomy: Classification and Nomenclature of Viruses. Eighth Report of the International Committee on the Taxonomy of Viruses, Elsevier, San Francisco, USA, pp. 571–580.

KOIWAI K., KODERA T., THAWONSUWAN J., RIANI S., KAWASE M., KONDO H. & HIRONO I. (2018). Rapid diagnosis of three shrimp RNA viruses using RT-PCR-DNA chromatography. *J. Fish Dis.,* 2018 May 28. doi: 10.1111/jfd.12821. Epub ahead of print. PMID: 29806113.

KUNANOPPARAT A., CHAIVISUTHANGKURA P., SENAPIN S., LONGYANY S., RUKPRATANPORN S., FLEGEL T.W. & SITHIGORNGUL P. (2011). Detection of infectious myonecrosis virus using monoclonal antibody specific to N and C fragments of the capsid protein expressed heterologously. *J. Virol. Methods*, **171**, 141–148.

LEE C.S. & O'BRYEN P.J., EDITORS (2003). Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables. World Aquaculture Society, Baton Rouge, LA, USA, 293 p.

LIGHTNER D.V. (2005). Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *J. World Aquac. Soc*., **36**, 229–248.

LIGHTNER D.V. (2011). Virus diseases of farmed shrimp in the Western Hemisphere (the Americas): a review. *J. Invertebr. Pathol*., **106,** 110–130.

LIGHTNER D.V., PANTOJA C.R., POULOS B.T., TANG K.F.J., REDMAN R.M., PASOS DE ANDRADE T. & BONAMI J.R. (2004). Infectious myonecrosis: new disease in Pacific white shrimp. *Global Aquaculture Advocate,* **7**, 85.

LIGHTNER D.V., REDMAN R.M., ARCE S. & MOSS S.M. (2009). Specific pathogen-free shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans. *In:* Shellfish Safety and Quality, Shumway S. & Rodrick G., eds. Woodhead Publishers, London, UK, pp. 384–424.

LOY D.S., LIU S., MOGLER M.A., LOY D.J., BLITVICH B.J. & BARTHOLOMAY L.C. (2015). Characterization of newly revealed sequences in the infectious myonecrosis virus genome in *Litopenaeus vannamei*. *J. Gen. Virol*., 96 (Pt **7**), 1821–1819.

MAI H.N., HANGGONO B., CARO L.F.A., KOMARUDDIN U., NUR'AINI Y.L.& DHAR A.K. (2019). Novel infectious myonecrosis virus (IMNV) genotypes associated with disease outbreaks on Penaeus vannamei shrimp farms in Indonesia. *Arch. Virol.,* **164**, 3051–3057. doi: 10.1007/s00705-019-04408-5. Epub 2019 Sep 17. PMID: 31531743.

MOSS S.M. & MOSS D.R. (2009). Chapter 17: Selective breeding of penaeid shrimp. *In:* Shellfish Safety and Quality, Shumway S. & Rodrick G., eds. Woodhead Publishers, London, UK. pp. 425–452.

NAIM S., BROWN J.K. & NIBERT M.L. (2014). Genetic diversification of penaeid shrimp infectious myonecrosis virus between Indonesia and Brazil. *Virus Res*., **189**, 99–105.

NAIM S., TANG K.F.J., YANG M., LIGHTNER D.V. & NIBERT M.L. (2015). Extended genome sequences of penaeid shrimp infectious myonecrosis virus strains from Brazil and Indonesia. *Arch. Virol*., **160**, 1579–1583.

NIBERT M.L. (2007). '2A-like' and 'shifty heptamer' motifs in penaeid shrimp infectious myonecrosis virus, a monosegmented double-stranded RNA virus. *J. Gen. Virol.*, **88**, 1315–1318.

NUNES A.J.P., CUNHA-MARTINS P. & VASCONSELOS-GESTEIRA T.C. (2004). Carcinicultura ameaçada. *Rev. Panoram. Aquic*., **83**, 37–51 (in Portuguese).

POULOS B.T. & LIGHTNER D.V. (2006). Detection of infectious myonecrosis virus (IMNV) of penaeid shrimp by reverse-transcriptase polymerase chain reaction (RT-PCR). *Dis. Aquat. Org*., **73**, 69–72.

POULOS B.T., TANG K.F.J., PANTOJA C.R., BONAMI J.R. & LIGHTNER D.V. (2006). Purification and characterization of infectious myonecrosis virus of penaeid shrimp. *J. Gen. Virol.*, **87**, 987–996.

SAHUL HAMEED A.S., ABDUL MAJEED S., VIMAL S., MADAN N., RAJKUMAR T., SANTHOSHKUMAR S. & SIVAKUMAR S. (2017). Studies on the occurrence of infectious myonecrosis virus in pond-reared *Litopenaeus vannamei* (Boone, 1931) in India. *J. Fish Dis.,* **40**, 1823–1830. doi: 10.1111/jfd.12655. Epub 2017 Jun 20. PMID: 28631825

SANTHOSH KUMAR S., SIVAKUMAR S., ABDUL MAJEED S., VIMAL S., TAJU G. & SAHUL HAMEED A.S. (2021). *In vitro* propagation of infectious myonecrosis virus in C6/36 mosquito cell line. *J. Fish Dis.,* **44**, 987–992. doi: 10.1111/jfd.13359. Epub 2021 Feb 25. PMID: 33631045.

SENAPIN S., PHEWSAIYA K., BRIGGS M. & FLEGEL T.W. (2007). Outbreaks of infectious myonecrosis virus (IMNV) in Indonesia confirmed by genome sequencing and use of an alternative RT-PCR detection method. *Aquaculture*, **266**, 32–38.

TANG K.F.J., PANTOJA C.R., POULOS B.T., REDMAN R.M. & LIGHTNER D.V. (2005). *In situ* hybridization demonstrates that *Litopenaeus vannamei, L. stylirostris* and *Penaeus monodon* are susceptible to experimental infection with infectious myonecrosis virus (IMNV). *Dis. Aquat. Org*., **63**, 261–265.

TANG K.F.J., PANTOJA C.R., REDMAN R.M. & LIGHTNER D.V. (2007). Development of *in situ* hybridization and RT-PCR assay for the detection of a nodavirus (*Pv*NV) that causes muscle necrosis in *Penaeus vannamei*. *Dis. Aquat. Org*., **75**, 183–190.

VANPATTEN K.A., NUNAN L.M. & LIGHTNER D.V. (2004). Seabirds as potential vectors of penaeid shrimp viruses and the development of a surrogate laboratory model utilizing domestic chickens. *Aquaculture*, **241**, 31–46.

VIEIRA-GIRÃO P.R.N., FALCÃO C.B., ROCHA I.R.C.B., LUCENA H.M.R., COSTA F.H.F. & RÁDIS-BAPTISTA G. (2017). Antiviral Activity of Ctn[15–34], A Cathelicidin-Derived Eicosapeptide, Against Infectious Myonecrosis Virus in *Litopenaeus vannamei* Primary Hemocyte Cultures. *Food Environ. Virol*., **9**, 277–286. doi: 10.1007/s12560-017-9285-5. Epub 2017 Feb 16. PMID: 28210987.

WHITE-NOBLE B.L., LIGHTNER D.V., TANG K.F.J. & REDMAN R. (2010). Lab challenge for selection of IMNV-resistant white shrimp. *Global Aquaculture Advocate*, July/August, 71–73.

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NB: At the time of publication (2022) there was no OIE Reference Laboratory for infection with infectious myonecrosis virus (please consult the OIE web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

NB: FIRST ADOPTED IN 2009. MOST RECENT UPDATES ADOPTED IN 2017.

CHAPTER 2.2.7.

INFECTION WITH TAURA SYNDROME VIRUS

1. Scope

Infection with Taura syndrome virus means infection with the pathogenic agent Taura syndrome virus (TSV), Genus Aparavirus, Family *Dicistroviridae*, Order Picornavirales.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

TSV was described as the cause of the disease commonly known as Taura syndrome by Hasson *et al.* (1995), Bonami *et al.* (1997) and Mari *et al.* (1998; 2002). At least four genotypes (strains) of TSV have been documented based on the gene sequence encoding VP1 the largest and presumably dominant of the three major structural proteins of the virus. Based on VP1 sequence variations, these genotypic groups are: 1) the Americas group; 2) the South-East Asian group; 3) the Belize group; and 4) the Venezuelan group (Nielsen *et al.,* 2005; Tang & Lightner, 2005; Wertheim *et al.,* 2009).

At least two distinct antigenic variants of TSV have been identified by their differential reactivity to monoclonal antibody MAb 1A1, produced using a reference isolate from the Americas (TSV USA-HI94 – GenBank AF277675) (Poulos *et al.,* 1999) as the immunogen: Type A represents those that react with MAb 1A1 (in the enzyme-linked immunosorbent assay [ELISA], Western blots and immunohistochemistry (IHC) with infected tissues) and those that do not were subdivided into Type B (TSV 98 Sinaloa, Mexico) and Type C (TSV 02 Belize), based on host species and virulence. All TSV isolates of the Americas and most, if not all, South-East Asian genotypes react with MAb 1A1. In marked contrast, none of the Belize genotype group reacts with MAb 1A1 (Robles-Sikisaka *et al.,* 2002), nor does a TSV isolate from the 2005 epizootic in Venezuelan shrimp farms.

TSV particles are 32 nm in diameter, non-enveloped icosahedrons and have a buoyant density of 1.338 g ml^{-1} in CsCl. The genome of TSV consists of a linear, positive-sense single-stranded RNA 10,205 nucleotides in length, excluding the 3' poly-A tail, and it contains two large open reading frames (ORFs). ORF 1 contains the sequence motifs for non-structural proteins, such as helicase, protease and RNA-dependent RNA polymerase. ORF 2 contains the sequences for TSV structural proteins, including the three major capsid proteins VP1, VP2 and VP3 (55, 40, and 24 kDa, respectively). The virus replicates in the cytoplasm of host cells (Bonami *et al.,* 1997; Mari *et al.,* 1998; 2002; Robles-Sikisaka *et al.,* 2001).

Other reported causes of Taura syndrome: TS in Ecuador was initially linked to fungicide contamination of shrimp farms, a contention that was supported by litigation for ~16 years after the disease was scientifically shown to have a viral aetiology (Brock *et al.,* 1995; Hasson *et al.,* 1995). Hence, several papers in the literature propose a toxic aetiology for TS (Intriago *et al.,* 1997; Jimenez, 1992; Jimenez *et al.,* 2000).

2.1.2. Survival and stability in processed or stored samples

No information available.

2.1.3. Survival and stability outside the host

No information available.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with TSV according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are: blue shrimp (*Penaeus stylirostris*), giant tiger prawn (*Penaeus monodon*), greasyback shrimp (*Metapenaeus ensis*), northern brown shrimp (*Penaeus aztecus*), northern white shrimp (*Penaeus setiferus*), and whiteleg shrimp (*Penaeus vannamei*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with TSV according to Chapter 1.5 of the *Aquatic Code* are: fleshy prawn (*Penaeus chinensis*), giant river prawn (*Macrobrachium rosenbergii*), the copepod *Ergasilus manicatus*, and the barnacles *Chelonibia patula* and *Octolasmis muelleri.* Evidence is lacking for these species to either confirm that the identity of the pathogenic agent is TSV, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: blue crab (*Callinectes sapidus*), the crabs *Uca vocans* and *Sesarma mederi*, gulf killifish (*Fundulus grandis*), Indo-Pacific swamp crab (*Scylla serrata*), kuruma prawn (*Penaeus japonicus*), northern pink shrimp (*Penaeus duorarum*) and southern white shrimp (*P. schmitti*).

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Infection with TSV has been documented in all life stages (i.e. post-larvae [PL], juveniles and adults) of *P. vannamei* except eggs, zygotes and larvae (Lightner, 1996a).

2.2.4. Distribution of the pathogen in the host

Using injection and *per os* challenge experiments, Nunan *et al.* (2004) demonstrated TSV could be detected in different body parts including gills, head, whole tail, tail muscle, pleopod and tail fan (Nunan *et al.,* 2004). While there was no significant difference in the viral copy number contained in different body parts when TSV was administered via injection, there was a statistically significant difference between tail/gills, tail/head, tail/tail fan, whole tail/tail fan and pleopods/tail fan when the viral inoculum was administered *per os*. The tail samples had the lower viral copy numbers, as did the whole tail and pleopods when compared to the tail fan (Nunan *et al.,* 2004).

2.2.5. Aquatic animal reservoirs of infection

Not demonstrated unequivocally

2.2.6. Vectors

Sea birds: TSV has been demonstrated to remain infectious for up to 48 hours (after ingestion of TSV-infected shrimp carcasses) in the faeces passed by wild or captive sea gulls (*Larus atricilla*) and chickens (*Gallus gallus,* used as a laboratory surrogate for all shrimp-eating birds) thus suggesting that the virus can retain infectivity when passed through the gastro-intestinal system of any bird species. These findings implicate birds as being an important mechanical vector for the transmission of the virus within affected farms or farming regions (Garza *et al.,* 1997; Vanpatten *et al.,* 2004).

Aquatic insects: the water boatman (*Trichocorixa reticulata* [*Corixidae*], an aquatic insect that feeds on shrimp carcasses in shrimp farm ponds) have been demonstrated to transport TSV within their intestinal contents, but are not directly infected by the virus (Brock, 1997; Lightner, 1996a; 1996b; reviewed in Dhar *et al.,* 2004).

2.3. Disease pattern

Infection with TSV is best known as a disease of nursery- or grow-out-phase *P. vannamei* that occurs within ~14–40 days of stocking PLs into grow-out ponds or tanks, hence, shrimp with TSV infection are typically small (~0.05 g to <5 g) juveniles. Larger shrimp may also be affected, especially if they are not exposed to the virus until they are larger juveniles or adults (Brock, 1997; Brock *et al.,* 1995; Lightner, 1996a, 1996b; Lotz, 1997).

2.3.1. Mortality, morbidity and prevalence

At the farm level, outbreaks of infection with TSV involving stocks of *P. vannamei* (the principal host species for infection with TSV) not selected for resistance, typical cumulative mortalities range from 40 to >90% in cultured populations of PL, juvenile, and subadult life stages. TSV-resistant lines of *P. vannamei* are available which show survival rates of up to 100% in laboratory challenge with all four TSV genotypes (Lightner *et al.,* 2009).

In regions where the virus is enzootic in farmed stocks, the prevalence of infection with TSV has been found in various surveys to range from 0 to 100% (Brock, 1997; Jimenez *et al.,* 2000).

2.3.2. Clinical signs, including behavioural changes

Only acute-phase clinical infection with TSV can be presumptively diagnosed from clinical signs. See Section 4.2 for a description of gross clinical signs presented by shrimp with acute-phase clinical infection with TSV.

Only shrimp with acute-phase clinical infection with TSV present behavioural changes. Typically, severely affected shrimp apparently become hypoxic and move to the pond edges or pond surface where dissolved oxygen levels are higher. Such shrimp may attract seabirds in large numbers. In many disease outbreaks, it is the large numbers of seabirds attracted to the moribund shrimp that first indicates the presence of a serious disease outbreak (which is often either infection with TSV or white spot syndrome virus) to the farm manager.

2.3.3. Gross pathology

Infection with TSV has three distinct phases, acute, transition, and chronic, which are grossly distinguishable (Hasson *et al.,* 1999a; 1999b; Lightner, 1996a; 1996b; Lightner *et al.,* 1995). Gross signs presented by juvenile, subadult and adult shrimp in the transition phase of infection with TSV are unique and provide a suspicion of infection.

Acute phase: gross signs displayed by moribund *P. vannamei* with acute-phase infection with TSV include expansion of the red chromatophores giving the affected shrimp a general, overall pale reddish colouration and making the tail fan and pleopods distinctly red; hence 'red tail' disease was one of the names given by farmers when the disease first appeared in Ecuador (Lightner *et al.,* 1995). In such shrimp, close inspection of the cuticular epithelium in thin appendages (such as the edges of the uropods or pleopods) with a ×10 hand lens reveals signs of focal epithelial necrosis. Shrimp showing these gross signs of acute infection with TSV typically have soft shells, an empty gut and are often in the late D stages of the moult cycle. Acutely affected shrimp usually die during ecdysis.

Transition (*recovery*) *phase:* although only present for a few days during outbreaks of infection with TSV, the gross signs presented by shrimp in the transition phase can provide a suspicion of infection with TSV. During the transition phase (which may be occurring while many shrimp in the affected populations are still in the acute phase and daily mortalities are high), fair to moderate numbers of shrimp in affected ponds show random, multifocal, irregularly shaped melanised cuticular lesions. These melanised spots are haemocyte accumulations indicating the sites of resolving TSV lesions in the cuticular epithelium. Such shrimp may or may not have soft cuticles and red-chromatophore expansion, and may be behaving and feeding normally (Brock, 1997; Hasson *et al.,* 1999b; Lightner, 1996a).

Chronic phase: after successfully moulting, shrimp in the transition phase move into the chronic phase of infection with TSV in which persistently infected shrimp show no obvious signs of disease (Brock, 1997; Hasson *et al.,* 1999b; Lightner, 1996a; 1996b; Lightner *et al.,* 1995). However, *P. vannamei* that are chronically infected with TSV may be less resistant to normal environmental stressors (i.e. sudden salinity reductions) than uninfected shrimp.

2.3.4. Modes of transmission and life cycle

Not applicable.

2.3.5. Environmental factors

Outbreaks of infection with TSV are more frequent when salinities are below 30 ppt (Jimenez *et al.,* 2000).

2.3.6. Geographical distribution

TSV is now widely distributed in the shrimp-farming regions of the Americas, South-East Asia and the Middle East (Brock, 1997; Hasson *et al.,* 1999a; Lightner, 1996a, 1996b; Lightner *et al.,* 2012; Lotz *et al.,* 2005; Nielsen *et al.,* 2005; Tang & Lightner, 2005; Tu *et al.,* 1999; Wertheim *et al.,* 2009; Vergel *et al.,* 2019; Yu & Song, 2000).

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No effective vaccines for TSV are available.

2.4.2. Chemotherapy including blocking agents

No scientifically confirmed reports of effective chemotherapy treatments.

2.4.3. Immunostimulation

No scientifically confirmed reports of effective immunostimulation treatments.

2.4.4. Breeding resistant strains

After TSV emerged in Ecuador in 1992–1994, *P. stylirostris* were found that possessed resistance to infection with TSV (genotype 1, MAb 1A1 Type A). Following on from this discovery and due to the disease occurrence in Mexico in 1994 where it caused crop failures of *P. vannamei,* selected lines of TSV-resistant *P. stylirostris* became the dominant shrimp farmed in western Mexico from 1995. However, in 1998–1999, a new 'strain' of TSV (Type B; Fegan & Clifford, 2001; Lightner, 1999; 2005; Zarain-Herzberg & Ascencio, 2001) emerged and caused massive epizootics in *P. stylirostris*. The emergence of this new 'strain' of TSV was soon followed in late 1999 by the introduction of white spot syndrome virus (WSSV) into shrimp farms in western Mexico, to which *P. stylirostris* had no resistance, effectively ending any interest in the culture of *P. stylirostris* in Mexico.

TSV-resistant domesticated stocks of *P. vannamei* and *P. stylirostris* have been developed. Some domesticated lines of TSV-resistant *P. vannamei* (that are also TSV-free) are in widespread use by the shrimp-farming industries of the Americas and South-East Asia (Clifford, 1998; White *et al.,* 2002). After the appearance of infection with TSV in Central America, improved TSV resistance was reported in wild caught *P. vannamei* PLs used to stock shrimp farms in the region. Currently all genetic lines of *P. vannamei* shrimp that are being cultured in Asia and the Americas contain varying levels of tolerance/resistance to TSV.

2.4.5. Inactivation methods

No information available.

2.4.6. Disinfection of eggs and larvae

It is possible that TSV might be transmitted vertically (transovarian transmission), despite the lack of published reports documenting this route of transmission. Disinfection of eggs and larvae (Chen *et al.,* 1992) is good management practice and it is recommended for its potential to reduce TSV contamination of spawned eggs and larvae produced from them.

2.4.7. General husbandry

Some husbandry and disease control and management practices have been used successfully to reduce the risks of infection with TSV occurring during farm grow-out. These include the application of PCR assays for pre-screening of wild or pond-reared broodstock or their spawned eggs/nauplii and discarding those that test positive for the virus (Fegan & Clifford, 2001), fallowing and restocking of entire culture regions with TSV-free stocks (Dixon & Dorado, 1997), and the development of specific pathogen-free (SPF) shrimp stocks of *P. vannamei* and *P. stylirostris* (Lightner, 1996b; 2005; Wyban 1992). The adoption of the latter technology (SPF stocks) has proven to be among the most successful husbandry practice for the prevention and control of infection with TSV.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Suitable specimens for testing for infection with TSV include PL, juveniles and adults. While TSV may infect all life stages, infection severity, and hence virus load, may be below detection limits in spawned eggs and in the larval stages, so these life stages may not be suitable samples for TSV detection or certification of freedom from infection with TSV.

3.2. Selection of organs or tissues

TSV infects tissues of ectodermal and mesodermal origin. The principal target tissue in the acute phase of infection with TSV is the cuticular epithelium. In chronic infections the lymphoid organ (LO) is the principal target tissue.

Haemolymph or excised pleopods may be collected and used when non-lethal testing of valuable broodstock is necessary.

3.3. Samples or tissues not suitable for pathogen detection

TSV is a systemic virus, and it does not replicate in enteric tissues (e.g. the hepatopancreas, the midgut, or its caeca). Hence, enteric tissues are inappropriate samples for detection of infection with TSV.

3.4. Non-lethal sampling

Haemolymph or pleopods can be collected without sacrificing the animals and used as non-lethal sampling of genetically valuable broodstock.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0. *General information* (*diseases of crustaceans*).

3.5.1. Samples for pathogen isolation

The success of pathogen isolation depends strongly on the quality of samples (which is influenced by time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be preserved in ethanol it may be frozen.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Chapter 2.2.0. *General information* (*diseases of crustaceans*).

3.5.4. Samples for other tests

Haemolymph could be used for PCR-based detection of TSV.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be

suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger animals should be processed and tested individually.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability, cost, timeliness, sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts						+	+	NA				
Histopathology		+	+	NA	+	+	+	NA				
Cell culture												
Real-time RT-PCR	+++	+++	+++	1	+++	+++	+++	1	+++	+++	+++	1
Conventional RT-PCR	++	++	++	1	++	++	++	1				
Conventional RT-PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation					+	+	+	1	+	+	+	1
Bioassay					+	+	+	1				
LAMP												
IFAT												
ELISA												
Other antigen detection methods												
Other method												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); NA = not available; PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; IFAT = indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay, respectively.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6).
[2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Direct microscopy of simple unstained wet mounts from excised pieces of the gills, appendage tips, etc., examined by phase- or reduced-light microscopy may be used to demonstrate (and make a tentative diagnosis of acute-phase infection with TSV) focal lesions of acute-phase infection with TSV in cuticular epithelial cells. Preparations presenting acute-phase infection with TSV will contain numerous spherical structures (see the histopathological methods in Section 4.2.3 above), which are pyknotic and karyorrhectic nuclei and cytoplasmic remnants of necrotic cells.

4.2. Histopathology and cytopathology

Histopathology is a useful method to detect infection with TSV in the acute and chronic phases of infection (Hasson *et al.,* 1999b; Lightner, 1996a). In chronic infections with TSV, the only lesion typically presented by infected shrimp is the presence of an enlarged LO with multiple LO spheroids (LOS) (Hasson *et al.,* 1999b), which cannot be distinguished from LOS induced by chronic infections of other RNA viruses (Lightner, 1996a). When histological lesions are observed and infection with TSV is suspected, a molecular test (ISH with TSV-specific probes, or reverse-transcription [RT] PCR) must be used for confirmation of infection with TSV (see Section 6).

4.2.1. Acute phase of Taura syndrome

The acute phase of the disease is characterised by multifocal areas of necrosis in the cuticular epithelium of the general body surface, appendages, gills, hindgut, and foregut (the oesophagus, anterior and posterior chambers of the stomach). Cells of the subcuticular connective tissues and adjacent striated muscle fibres basal to affected cuticular epithelium are occasionally affected. In some severe cases of acute-phase infection with TSV, the antennal gland tubule epithelium is also destroyed. Prominent in the multifocal cuticular lesions are conspicuous foci of affected cells that display an increased eosinophilia of the cytoplasm and pyknotic or karyorrhectic nuclei. Cytoplasmic remnants of necrotic cells are often extremely abundant in these infections with TSV acute-phase lesions and these are generally presented as spherical bodies (1–20 μm in diameter) that range in staining from eosinophilic to pale basophilic. These structures, along with pyknotic and karyorrhectic nuclei, give acute-phase TS lesions a characteristic 'peppered' or 'buckshot-riddled' appearance, which is considered to be pathognomonic for the infection when there is no concurrent necrosis of the parenchymal cells of the LO tubules. The absence of necrosis of the LO in acute-phase infection with TSV distinguishes it from acute-phase infection with yellowhead virus genotype 1 in which similar patterns of necrosis to those induced by infection with TSV may occur in the cuticular epithelium and gills (Lightner, 1996a).

In TSV-infected tissues, pyknotic or karyorrhectic nuclei give a positive (for DNA) Feulgen reaction, which distinguishes them from the less basophilic to eosinophilic cytoplasmic inclusions that do not contain DNA. The absence of haemocytic infiltration or other signs of a significant host-inflammatory response distinguishes the acute phase of infection with TSV from the transitional phase of the disease (Brock, 1997; Brock *et al.,* 1995; Hasson *et al.,* 1995; 1999a; 1999b; Lightner, 1996a; Lightner *et al.,* 1995).

4.2.2. Transition (recovery) phase of infection with Taura syndrome virus

In the transitional phase of infection with TSV, typical acute-phase cuticular lesions decline in abundance and severity and are replaced by conspicuous infiltration and accumulation of haemocytes at the sites of necrosis. The masses of haemocytes may become melanised giving rise to the irregular black spots that characterise the transition phase of the disease. In H&E sections, such lesions may show erosion of the cuticle, surface colonisation and invasion of the affected cuticle and exposed surface haemocytes by *Vibrio* spp. (Hasson *et al.,* 1999b; Lightner, 1996a). Sections of the LO during the transition phase of infection with TSV may appear normal with H&E staining. However, when sections of the LO are assayed for TSV by ISH with a specific cDNA probe (or by ISH with MAb 1A1 for TSV type A, genotype 1), large quantities of TSV are shown accumulating in the more peripheral parenchymal cells of the LO tubules (Hasson *et al.,* 1999b; Srisuvan *et al.,* 2005).

4.2.3. Chronic phase of infection with Taura syndrome virus

Shrimp in the chronic phase of infection with TSV display no gross signs of infection, and histologically the only sign of infection is the presence of numerous prominent LOS, which may remain associated with the main body of the paired LO, or which may detach and become ectopic LOS bodies that lodge in constricted areas of the haemocoel (i.e. the heart, gills, in the subcuticular connective tissues, etc.). Such LOS are spherical accumulations of LO cells and haemocytes and may be distinguished from normal LO tissues by their spherical nature and the lack of the central vessel that is typical of normal LO tubules. When assayed by ISH with a cDNA

probe for TSV (or with MAb 1A1 using ISH) some cells in the LOS give positive reactions to the virus, while no other target tissues react (Hasson *et al.,* 1999b; Lightner, 1996a; 1996b).

4.3. Cell culture for virus isolation

TSV has not been grown *in vitro*, as no crustacean cell lines exist (Lightner, 1996a; Pantoja *et al.,* 2004). Although one publication incorrectly reported that TSV infected human and monkey cell lines (Audelo del Valle *et al.,* 2003), two other laboratories that repeated the study both found that TSV does not infect or replicate in primate or human cell lines that are known to have susceptibility to human picornaviruses (Luo *et al.,* 2004; Pantoja *et al.,* 2004).

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

4.4.1. Real-time reverse-transcription (RT)-PCR

Real-time RT-PCR methods have been developed for the detection of TSV. These methods have the advantage of speed, specificity and sensitivity. The sensitivity of real time RT-PCR is approximately equal to 100 copies of the target sequence from the TSV genome (Dhar *et al.,* 2002; Tang *et al.,* 2004).

The real-time RT-PCR method described below for TSV follows the method used in Tang *et al.,* 2004.

Primer and probe sequences, real time RT-PCR

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1 (Tang *et al.*,2004) GenBank Accession No. AFAF277675			
ORF-1 Nt 1024 to 1051	Fwd: TSV1004: TTG-GGC-ACC-AAA-CGA-CAT-T- Rev: TSV1075 GGG-AGC-TTA-AAC-TGG-ACA-CAC-TGT Probe: TSV-P1 FAM-CAG-CAC-TGA-CGC-ACA-ATA-TTC-GAG-CAT-C-TAMRA,	300 nM of each primer 100 nM of probe	Reverse transcription at 50°C/30 min 40 cycles of 95°C/3 sec and 60°C/30 sec

4.4.2. Conventional RT-PCR

Tissue samples (haemolymph, pleopods, whole small shrimp etc) may be assayed for TSV using RT-PCR. The RT-PCR method outlined below for TSV follows the method used in Nunan *et al.* (1998).

Primer and probe sequences, conventional RT-PCR

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1 (Nunan *et al.,* 1998); product size 231 bp			
ORF 2	Fwd: 9992: AAG-TAG-ACA-GCC-GCG-CTT Rev:9195R: TCA-ATG-AGA-GCT-TGG-TCC	Primers/620 nM each	Reverse transcription 60°C/30 min 40 cycles: 94°C/45 sec, 60°C/45 sec

4.4.3. Other nucleic acid amplification methods

None currently available.

4.5. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced and analysed and compared with published sequences.

4.6. *In-situ* hybridisation (ISH)

4.6.1. DNA probes for ISH applications with non-radioactive cDNA probes

Non-radioactive, DIG-labelled cDNA probes for detection of TSV may be produced in the laboratory. The ISH method provides greater diagnostic sensitivity than do more traditional methods for TSV detection and diagnosis that employ classic histological methods (Hasson *et al.,* 1999a; Lightner, 1996a; 1999; Lightner & Redman 1998; Mari *et al.,* 1998). The ISH assay of routine histological sections of acute- and transition-phase lesions in the cuticular epithelium, other tissues, and of LOS in transition and chronic phase with a specific DIG-labelled cDNA probe to TSV, provides a definitive diagnosis of infection with TSV (Hasson *et al.,* 1999a; 1999b; Lightner, 1996a; 1996b). Pathognomonic TSV-positive lesions display prominent blue to blue-black areas in the cytoplasm of affected cells when reacted with the cDNA probes. Not reacting to the probe are the prominent karyorrhectic nuclear fragments and pyknotic nuclei that contribute to the pathognomonic 'buckshot riddled' appearance of TS lesions (Lightner, 1996a; Mari *et al.,* 1998). (See Chapter 2.2.4 *Infection with infectious hypodermal and haematopoietic necrosis virus* for details of the ISH method, and Chapter 2.2.0 Section B.5.3.ii for detailed information on the use of Davidson's AFA fixative.)

False-negative ISH results may occur with Davidson's fixed tissues if tissues are left in fixative for more than 24–48 hours. The low pH of Davidson's fixative causes acid hydrolysis of the TSV single-stranded RNA genome, resulting in false-negative probe results. This hydrolysis can be prevented by avoiding fixation times over 24 hours (Hasson *et al.,* 1997; Lightner, 1996a; Lightner & Redman 1998).

4.7. Immunohistochemistry

Not suitable.

4.8. Bioassay

Confirmation of infection with TSV may be accomplished by bioassay of TSV-suspect animals with SPF juvenile *P. vannamei* serving as the indicator of the virus (Garza *et al.,* 1997; Hasson *et al.,* 1999b; 1995; Lightner, 1996a; Lotz, 1997; Overstreet *et al.,* 1997). Oral or injection protocols may be used. The oral method is relatively simple to perform and is accomplished by feeding chopped carcasses of suspect shrimp to SPF juvenile *P. vannamei* in small tanks (White *et al.,* 2002). The use of a negative control tank of indicator shrimp, which receive only SPF (TSV-free) tissue and normal shrimp feed is required. When the carcass feeding (*per os*) protocol is used to bioassay for TSV, TSV-positive indicator shrimp (by gross signs and histopathology) are typically apparent within 3–4 days of initial exposure, and significant mortalities occur by 3–8 days after initial exposure. The negative control shrimp must remain negative (for at least 10–15 days) for gross or histological signs of disease and unusual mortalities (Hasson *et al.,* 1999b; Lightner, 1996a; White *et al.,* 2002).

With the injection bioassay protocol, a variety of sample types may be tested for TSV. Whole shrimp are used if they were collected during an outbreak of infection with TSV. Heads only should be used if shrimp display gross transition-phase lesions (multifocal melanised spots on the cuticle) or no clinical signs of infection (chronic phase) as the virus, if present, will be concentrated in the LO (Hasson *et al.,* 1999b; Lightner, 1996a). For non-lethal testing of broodstock, haemolymph samples may be taken and used to expose the indicator shrimp by IM injection (Lightner, 1996a).

4.9. Antibody- or antigen-based detection methods

Not recommended.

4.10. Other methods

4.10.1. Dot-blot immunoassay method

i) For the dot-blot immunoassay method, 1 µl of test antigen (purified virus, infected shrimp haemolymph or SPF shrimp haemolymph) is dotted on to the surface of MA-HA-N45 assay plates (Millipore) [10].

ii) After air drying, the wells are blocked for 1 hour at room temperature with 200 µl of a buffer containing phosphate-buffered saline and 0.05% Tween 20 (PBST) mixed with 10% normal goat serum (Life Technologies) and 2% Hammersten casein (Amersham Life Sciences).

iii) The wells are washed three times with PBST and then reacted with 100 µl primary antibody (MAb or mouse polyclonal antibodies) for 30 minutes at room temperature.

iv) Alkaline-phosphatase-labelled goat anti-mouse IgG, γ chain specific, secondary antibody (Zymed) diluted 1/1000 in PBST plus 10% normal goat serum is used for detection (30 minutes at room temperature).

v) After washing three times with PBST, once with PBS and once with distilled water, the reactions are visualised by development for 15 minutes at room temperature with nitroblue tetrazolium and bromo-chloro-indoyl phosphate (Roche Diagnostics in 100 mM Tris-HCl, 100 mM NaCl buffer containing 50 mM $MgCl_2$, pH 9.5.

vi) Reactions are stopped with distilled water.

vii) The reactions are graded using a scale from 0 to +4, with the highest intensity reaction being equivalent to the reaction generated using the MAb against the reference control consisting of semi-purified TSV. A negative reaction is one in which no coloured spot is visible in the well.

5. Test(s) recommended for surveillance to demonstrate disease freedom in apparently healthy populations

Real-time RT-PCR is the recommended test for surveillance to demonstrate freedom of infection with TSV in apparently healthy populations as described in Section 4.1.1.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (6.1) or presence of clinical signs (6.2) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision-making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [11]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with TSV shall be suspected if at least one of the following criteria is met:

i) Histopathological changes consistent with the presence of the pathogen or the disease

ii) A positive result by real-time RT-PCR

10 Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

11 For example transboundary commodities.

iii) A positive result by conventional RT-PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with TSV is considered to be confirmed if at least one of the following criteria is met:

i) A positive result by real-time RT-PCR and a positive result by conventional RT-PCR followed by sequencing of the amplicon

ii) A positive result by *in-situ* hybridisation and a positive result by real-time RT-PCR

iii) A positive result by *in-situ* hybridisation and a positive result by conventional RT-PCR followed by amplicon sequencing

6.2. Clinically affected animals

No clinical signs are pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with TSV shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Histopathological changes consistent with TSV infection

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

v) Positive result of a bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with TSV is considered to be confirmed if at least at least one of the following criteria is met:

i) A positive result by real-time RT-PCR and a positive result by conventional RT-PCR followed by sequencing of the amplicon

ii) A positive result by *in-situ* hybridisation and a positive result by real-time RT-PCR

iii) A positive result by *in-situ* hybridisation and a positive result by conventional RT-PCR followed by amplicon sequencing

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with TSV are provided in Table 6.3.1 (none currently available). This information can be used for the design of surveys for infection with TSV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For surveillance of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

7. References

AUDELO DEL VALLE J., CLEMENT-MELLADO O., MAGANA-HERNANDEZ A., FLISSER A., MONTIEL-AGUIRRE F. & BRISENO-GARCIA B. (2003). Infection of cultured human and monkey cell lines with extract of penaeid shrimp infected with Taura syndrome virus. *Emerg. Infect. Dis.,* **9**, 265–266.

BONAMI J.R., HASSON K.W., MARI J., POULOS B.T. & LIGHTNER D.V. (1997). Taura syndrome of marine penaeid shrimp: characterization of the viral agent. *J. Gen. Virol.,* **78**, 313–319.

BROCK J.A. (1997). Special topic review: Taura syndrome, a disease important to shrimp farms in the Americas. *World J. Microbiol Biotechnol.,* **13**, 415–418.

BROCK J.A., GOSE R., LIGHTNER D.V. & HASSON K.W. (1995). An overview on Taura syndrome, an important disease of farmed *Penaeus vannamei*. *In:* Swimming through Troubled Water, Proceedings of the Special Session on Shrimp Farming, Aquaculture '95, Browdy C.L. & Hopkins J.S., eds. San Diego, California, 1–4 February 1995. World Aquaculture Society, Baton Rouge, Louisiana, USA, 84–94.

CHEN S.N., CHANG P.S. & KOU G.H. (1992). Infection route and eradication of *Penaeus monodon* baculovirus (MBV) in larval giant tiger prawns, *Penaeus monodon. In:* Diseases of Cultured Penaeid Shrimp in Asia and the United States, Fulks W. & Main K.L., eds. Oceanic Institute, Honolulu, Hawaii, USA, 177–184.

CLIFFORD H.C. (1998). Management of ponds stocked with blue shrimp *Litopenaeus stylirostris*. *In:* Proceedings of the First Latin American Shrimp Farming Congress, D.E. Jory, ed. Panama City, Panama, 1–11.

DHAR A.K., COWLEY J.A., HASSON K.W. & WALKER P.J. (2004). Taura syndrome virus and yellow head virus of penaeid shrimp. *Adv. Virus Res.,* 64, 353–421.

DIXON H. & DORADO J. (1997). Managing Taura syndrome in Belize: a case study. *Aquaculture Magazine*, May/June, 30–42.

FEGAN D.F. & CLIFFORD H.C. III. (2001). Health management for viral diseases in shrimp farms. *In:* The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture. Aquaculture 2001, Browdy C.L. & Jory D.E., eds. The World Aquaculture Society, Baton Rouge, Louisiana, USA, 168–198.

GARZA J.R., HASSON K.W., POULOS B.T., REDMAN R.M., WHITE B.L. & LIGHTNER D.V. (1997). Demonstration of infectious taura syndrome virus in the feces of sea gulls collected during an epizootic in Texas. *J. Aquat. Anim. Health,* **9**, 156–159.

HASSON K.W., HASSON J., AUBERT H., REDMAN R.M. & LIGHTNER D.V. (1997). A new RNA-friendly fixative for the preservation of penaeid shrimp samples for virological detection using cDNA genomic probes. *J. Virol. Methods,* **66**, 227–236.

HASSON K.W., LIGHTNER D.V., MARI J. & BONAMI J.R., POULOS B.T., MOHNEY L.L., REDMAN R.M. & BROCK J.R. (1999a). The geographic distribution of Taura Syndrome Virus (TSV) in the Americas: determination by histology and *in situ* hybridization using TSV-specific cDNA probes. *Aquaculture*, **171**, 13–26.

HASSON K.W., LIGHTNER D.V., MOHNEY L.L., REDMAN R.M., POULOS B.T. & WHITE B.M. (1999b). Taura syndrome virus (TSV) lesion development and the disease cycle in the Pacific white shrimp *Penaeus vannamei*. *Dis. Aquat. Org.,* **36**, 81–93.

HASSON K.W., LIGHTNER D.V., POULOS B.T., REDMAN R.M., WHITE B.L., BROCK J.A. & BONAMI J.R. (1995). Taura syndrome in *Penaeus vannamei*: demonstration of a viral etiology. *Dis. Aquat. Org.,* **23**, 115–126.

INTRIAGO P., JIMENEZ R., MACHUCA M., BARNIOL R., KRAUSS E. & SALVADOR X. (1997). Experiments on toxicosis as the cause of Taura Syndrome in *Penaeus vannamei* (Crustacea: Decapoda) in Ecuador. *In:* Diseases in Asian Aquaculture III, Flegel T.W. & MacRae I.H., eds. Fish Health Section, Asian Fisheries Society, Manila, the Philippines, 365–379.

JIMENEZ R. (1992). Sindrome de Taura (Resumen). *In:* Acuacultura del Ecuador. Camara Nacional de Acuacultura, Guayaquil, Ecuador, 1–16.

JIMENEZ R., BARNIOL R., DE BARNIOL L. & MACHUCA M. (2000). Periodic occurrence of epithelial viral necrosis outbreaks in *Penaeus vannamei* in Ecuador. *Dis. Aquat. Org.,* **42**, 91–99.

LIGHTNER D.V. (ED.) (1996a). A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA, 304 pp.

LIGHTNER D.V. (1996b). Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas. *Rev. sci. tech. Office int. Epiz*., **15**, 579–601.

LIGHTNER D.V. (1999). The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods and management strategies. *J. Appl. Aquac.*, **9**, 27–52.

LIGHTNER D.V. (2005). Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *J. World Aquac. Soc.,* **36**, 229–248.

LIGHTNER D.V. & REDMAN R.M. (1998). Shrimp diseases and current diagnostic methods. *Aquaculture*, **164**, 201–220.

LIGHTNER D.V., REDMAN R.M., ARCE S. & MOSS S.M. (2009). Specific pathogen-free shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans. *In:* Shellfish Safety and Quality, Shumway S. & Rodrick G., eds. Woodhead Publishers, London, UK. pp. 384-424.

LIGHTNER D.V., REDMAN R.M., HASSON K.W. & PANTOJA C.R. (1995). Taura syndrome in *Penaeus vannamei* (Crustacea: Decapoda): gross signs, histopathology and ultrastructure. *Dis. Aquat. Org.,* **21**, 53–59.

LIGHTNER D.V., REDMAN R.M., PANTOJA C.R., TANG K.F.J., NOBLE B.L., SCHOFIELD P., MOHNEY L.L., NUNAN L.M. & NAVARRO S.A. (2012). Historic emergence, impact and current status of shrimp pathogens in the Americas. *J. Invertebr. Pathol.,* **110**, 174–183.

LOTZ J.M. (1997). Effect of host size on virulence of Taura virus to the marine shrimp *Penaeus vannamei* (Crustacea: Penaeidae). *Dis. Aquat. Org.,* **30**, 45–51.

LOTZ J.M., ANTON, L.S. & SOTO M.A. (2005). Effect of chronic Taura syndrome virus infection on salinity tolerance of *Litopenaeus vannamei*. *Dis. Aquat. Org.,* **65**, 75–78.

LUO P., HU C.Q., REN C.H. & SUN Z.F. (2004). Taura syndrome virus and mammalian cell lines. *Emerg. Infect. Dis.,* **10**, 2260–2261.

MARI J., BONAMI J.R. & LIGHTNER D.V. (1998). Taura syndrome of penaeid shrimp: cloning of viral genome fragments and development of specific gene probes. *Dis. Aquat. Org.,* **33**, 11–17.

NAVARRO S.A., TANG K.F.J & LIGHTNER D.V. (2009). An improved Taura syndrome virus (TSV) RT-PCR using newly designed primers. *Aquaculture*, **293**, 290–292.

NIELSEN L., SANG-OUM W., CHEEVADHANARAK S. & FLEGEL T.W. (2005). Taura syndrome virus (TSV) in Thailand and its relationship to TSV in China and the Americas. *Dis Aquat. Org,* **63**, 101–106.

NUNAN L.M., POULOS B.T. & LIGHTNER D.V. (1998). Reverse transcription polymerase chain reaction (RT-PCR) used for the detection of Taura Syndrome Virus (TSV) in experimentally infected shrimp. *Dis. Aquat. Org.,* **34**, 87–91.

NUNAN L.M., TANG-NELSON K. & LIGHTNER D.V. (2004). Real-time RT-PCR determination of viral copy number in *Penaeus vannamei* experimentally infected with Taura syndrome virus. *Aquaculture*, **229**, 1–10.

OVERSTREET R.M., LIGHTNER D.V., HASSON K.W., MCILWAIN S. & LOTZ J. (1997). Susceptibility to TSV of some penaeid shrimp native to the Gulf of Mexico and southeast Atlantic Ocean. *J. Invertebr. Pathol.,* **69**, 165–176.

PANTOJA C.R., NAVARRO S.A., NARANJO J., LIGHTNER D.V. & GERBA C.P. (2004). Nonsusceptibility of primate cells to Taura syndrome virus. *Emerg. Infect. Dis.,* **10**, 2106–2112.

POULOS B.T., KIBLER R., BRADLEY-DUNLOP D., MOHNEY L.L. & LIGHTNER D.V. (1999). Production and use of antibodies for the detection of Taura syndrome virus in penaeid shrimp. *Dis. Aquat. Org.,* **37**, 99–106.

ROBLES-SIKISAKA R., HASSON K.W., GARCIA D.K., BROVONT K., CLEVELAND K., KLIMPEL K.R. & DHAR A.K. (2002). Genetic variation and immunohistochemical differences among geographical isolates of Taura syndrome virus of penaeid shrimp. *J. Gen. Virol.,* **83**, 3123–3130.

ROBLES-SIKISAKA R., GARCIA D.K., KLIMPEL K.R. & DHAR A.K. (2001). Nucleotide sequence of 3'-end of the genome of Taura syndrome virus of shrimp suggests that it is related to insect picornaviruses. *Arch. Virol.,* **146**, 941–952.

SRISUVAN T., TANG K.F.J. & LIGHTNER D.V. (2005). Experimental infection of *Penaeus monodon* with Taura syndrome virus (TSV). *Dis. Aquat. Org*., **67**, 1–8.

TANG K.F.J. & LIGHTNER D.V. (2005). Phylogenetic analysis of Taura syndrome virus isolates collected between 1993 and 2004 and virulence comparison between two isolates representing different genetic variants. *Virus Res.*, **112**, 69–76.

TANG K.F.J., WANG J. & LIGHTNER D.V. (2004). Quantitation of Taura syndrome virus by real-time RT-PCR with a TaqMan assay. *J. Virol. Methods,* **115**, 109–114.

TU C., HUANG H.T., CHUANG S.H., HSU J.P., KUO S.T., LI N.J., HUS T.L., LI M.C. & LIN S.Y. (1999). Taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. *Dis. Aquat. Org.,* **38**, 159–161.

VANPATTEN K.A., NUNAN L.M. & LIGHTNER D.V. (2004). Seabirds as potential vectors of penaeid shrimp viruses and the development of a surrogate laboratory model utilizing domestic chickens. *Aquaculture*, **241**, 31–46.

VERGEL J.C.V., CABAWATAN L.D.P., MADRONA V.A.C., ROSARIO A.F.T., STA. ANA J.B.M., TARE M.V.R. & MANINGAS M.B.B. (2019). Detection of Taura Syndrome Virus (TSV) in *Litopenaeus vannamei* in the Philippines. *Philipp. J. Fish*., **26**, 8–14.

WERTHEIM J.O., TANG K.F.J., NAVARRO S.A. & LIGHTNER D.V. (2009). A quick fuse and the emergence of Taura syndrome virus. *Virology,* **390**, 324–329.

WHITE B.L., SCHOFIELD P.J., POULOS B.T. & LIGHTNER D.V. (2002). A laboratory challenge method for estimating Taura Syndrome virus resistance in selected lines of Pacific White Shrimp *Penaeus vannamei*. *J. World Aquac. Soc*., **33**, 341–348.

WYBAN J.A. (1992). Selective breeding of specific pathogen-free (SPF) shrimp for high health and increased growth. *In:* Diseases of Cultured Penaeid Shrimp in Asia and the United States, Fulks W. & Main K.L., eds. The Oceanic Institute, Honolulu, Hawaii, USA, 257–268.

YU C.I. & SONG Y.L. (2000). Outbreaks of Taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. *Fish Pathol.,* **5**, 21–24.

ZARAIN-HERZBERG M. & ASCENCIO F. (2001). Taura syndrome in Mexico: follow-up study in shrimp farms of Sinaloa. *Aquaculture,* **193**, 1–9.

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NB: There is an OIE Reference Laboratory for infection with Taura syndrome virus
(please consult the OIE Web site for the most up-to-date list:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact OIE Reference Laboratories for any further information on
infection with Taura syndrome virus

NB: FIRST ADOPTED IN 2006. MOST RECENT UPDATES ADOPTED IN 2017.

CHAPTER 2.2.8.

INFECTION WITH WHITE SPOT SYNDROME VIRUS

1. Scope

Infection with white spot syndrome virus means infection with the pathogenic agent white spot syndrome virus (WSSV), Genus *Whispovirus,* Family *Nimaviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

WSSV was assigned by the International Committee on Taxonomy of Viruses (ICTV) as the only member of the genus *Whispovirus* within the *Nimaviridae* family. Virions of WSSV are ovoid or ellipsoid to bacilliform in shape, have a regular symmetry, and measure 80–120 nm in diameter and 250–380 nm in length. A flagella-like extension (appendage) may be observed at one end of the virion. WSSV has been divided into three groups: isolates originating from Thailand (WSSV-TH-96-II), isolates originating from India (WSSV-IN-07-I), and another Indian isolate (WSSV-IN-06-I). Most strains of WSSV were speculated to have originated from the Indian Ocean and then spread across the world (Zeng, 2021). Today, although various geographical isolates with genotypic variability have been identified, they are all classified as a single species (WSSV) within the genus *Whispovirus* (Lo *et al.,* 2012; Wang *et al*., 2019).

2.1.2. Survival and stability in processed or stored samples

Viable WSSV was found in frozen commodity shrimp imported to Australia from Southeast Asia (McColl *et al.,* 2004). The virulence of WSSV was retained for 14 months at –80°C in a filtered tissue homogenate prepared from moribund shrimp with hepatopancreas and abdomen removed (Momoyama *et al.,* 1998). The virus originally collected from the haemolymph of moribund shrimp could maintain its virulence for at least 16 months at –80°C (Wu *et al.,* 2002). However, WSSV might be inactivated by multiple freeze-thaw cycles due to damage the viral envelopes or nucleocapsids (Durand *et al.,* 2000; Hasson *et al.,* 2006).

2.1.3. Survival and stability outside the host

The agent is viable for at least 30 days at 30°C in seawater under laboratory conditions (Momoyama *et al*., 1998); and is viable in ponds for at least 3–4 days (Nakano *et al*., 1998). Laboratory emulations of drainable and non-drainable ponds suggest that the virus is no longer infective after 21 days of sun-drying or after 40 days in waterlogged pond sediment (Satheesh Kumar *et al*., 2013).

WSSV with an initial viral load of 1000 virions ml^{-1} was found to be viable for a period of 12 days in seawater of 27 ppt salinity, pH of 7.5 at 29–33°C. In shrimp pond sediment (with initial viral load of 211,500 copies g^{-1}), the virus was viable and infective up to 19 days despite sun-drying. In the case of non-drainable conditions, WSSV (753,600 copies g^{-1}) remained infective for a period of 35 days (Satheesh Kumar *et al*., 2013).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Of all the species that have been tested to date, no decapod (order Decapoda) crustacean from marine, brackish or freshwater sources has been reported to be refractory to infection with WSSV (Flegel, 1997; Lightner, 1996; Lo & Kou, 1998; Maeda *et al*., 2000; Stentiford *et al.,* 2009).

2.2.2. Species with incomplete evidence for susceptibility

All life stages are potentially susceptible, from eggs to broodstock (Lightner, 1996; Venegas *et al.,* 1999). WSSV genetic material has been detected in reproductive organs (Lo *et al.,* 1997), but susceptibility of the gametes to WSSV infection has not been determined definitively.

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

The best life stages of crustaceans for detection of WSSV are late postlarvae (PL) stages, juveniles and adults. Probability of detection can be increased by exposure to stressful conditions (e.g. eye-stalk ablation, spawning, moulting, changes in salinity, temperature or pH, and during plankton blooms).

2.2.4. Distribution of the pathogen in the host

The major target tissues of WSSV are of ectodermal and mesodermal embryonic origin, especially the cuticular epithelium and subcuticular connective tissues (Momoyama *et al*., 1994; Wongteerasupaya *et al*., 1995). Although WSSV infects the underlying connective tissue in the crustacean hepatopancreas and midgut, the tubular epithelial cells of these two organs are of endodermal origin, and they do not become infected.

2.2.5. Aquatic animal reservoirs of infection

Wild decapods known to be reservoirs of infection with WSSV include *Mysis* sp. (Huang *et al.,* 1995), *Acetes* sp., *Alpheus* sp., *Callianassa* sp., *Exopalaemon* sp., *Helice* sp., *Hemigrapsus* sp. *Macrophthalmus* sp., *Macrophthel* sp., *Metaplax* sp., *Orithyia* sp., *Palaemonoidea* sp., *Scylla* sp., *Sesarma* sp., and *Stomatopoda* sp. (Desrina *et al*., 2022; He & Zhou, 1996; Lei *et al.,* 2002). These species can express the disease under suitable environmental conditions. However, non-decapodal crustaceans, such as copepods (Huang *et al.,* 1995), rotifers (Yan *et al.,* 2004), *Balanus* sp. (Lei *et al.,* 2002), *Artemia* (Li *et al*., 2004; Zhang *et al*., 2010) and *Tachypleidue* sp. (He & Zhou, 1996) may be apparently healthy carrier animals. Other marine molluscs, polychaete worms (Vijayan *et al.,* 2005), as well as non-crustacean aquatic arthropods such as sea slaters (*Isopoda*), and Euphydradae insect larvae can mechanically carry the virus without evidence of infection (Lo & Kou, 1998).

2.2.6. Vectors

The harpacticoid copepod *Nitocra* sp. (Zhang *et al*., 2008), microalgae (Liu *et al*., 2007), and the polychaete, *Dendronereis* spp. (Peters) (Desrina *et al*., 2013; Haryadi *et al.,* 2015) are vectors for WSSV.

2.3. Disease pattern

Infection with WSSV sometimes causes clinical disease (Tsai *et al.*, 1999), depending on factors that are poorly understood but related to species tolerance and environmental triggers. With an appropriate infection dose to allow sufficient time before mortality, animals susceptible to disease show large numbers of virions circulating in the haemolymph (Lo *et al*., 1997), but this may also occur for tolerant species that show no mortality. Thus, high viral loads *per se* do not cause disease or mortality for all susceptible species.

2.3.1. Mortality, morbidity and prevalence

All penaeid shrimp species are highly susceptible to infection with WSSV, often resulting in high mortality. Crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters are susceptible to infection with WSSV, but morbidity and mortality as a consequence of infection are highly variable (Lo & Kou, 1998). High level infections with WSSV are known in some decapods in the absence of clinical disease.

Prevalence of infection with WSSV is highly variable, from <1% in infected wild populations to up to 100% in captive populations (Lo & Kou, 1998).

2.3.2. Clinical signs, including behavioural changes

White spots embedded within the exoskeleton are the most commonly observed clinical sign. In most shrimp, these spots range from barely visible to 3 mm in diameter, and they sometimes coalesce into larger plates.

However, it should be noted that environmental stress factors, such as high alkalinity, or bacterial disease can also cause white spots on the carapace of shrimp, and that moribund shrimp with infection with WSSV may have few, if any, white spots. Therefore, the appearance of white spots is not a reliable diagnostic sign of infection with WSSV infection. High degrees of colour variation with a predominance of reddish or pinkish discoloured shrimp are seen in diseased populations.

WSSV infections can be subclinical or manifest as clinical disease. The penaeid shrimp in aquaculture will generally show clinical signs associated with high morbidity and mortality. Some animals may die without showing any clinical signs. Non-penaeid species (e.g. crab, lobster) generally have subclinical infections under natural conditions.

The affected animals can show lethargy, decreased or absent feed consumption and abnormal swimming behaviour – slow swimming, swimming on side, swimming near water surface and gathering around edges of rearing units (Corbel *et al.,* 2001; Sahul Hameed *et al.,* 1998; 2001). A very high mortality rate in the shrimp population can be expected within a few days of the onset of behavioural signs.

2.3.3 Gross pathology

In addition to the clinical and behavioural signs in Section 2.3.2. above, the following gross pathology has been reported in clinically affected penaeid shrimp: loosened attachment of the carapace with underlying cuticular epithelium (Sanchez-Paz, 2010), so that the carapace can be easily removed (Zhan *et al.,* 1998); empty gastro-intestinal tract due to anorexia (Escobedo-Bonilla, 2008); delayed clotting of haemolymph (Heidarieh *et al.,* 2013); excessive fouling of gills (Wu *et al.,* 2013) and exoskeleton.

2.3.4. Modes of transmission and life cycle

Infection with WSSV can be transmitted horizontally by consumption of infected tissue (e.g. cannibalism, predation, fomites, etc.), by water-borne routes, and by other routes of transmission (e.g. via sea birds, anthropogenic movements, feeding, rotifer, copepods, etc) (Haryadi *et al.,* 2015; Vanpatten *et al.,* 2004; Zhang *et al.,* 2006; 2008). Transmission of WSSV can occur from apparently healthy animals in the absence of disease. Dead and moribund animals can be a source of disease transmission (Lo & Kou, 1998). Microalgae could serve as a potential horizontal transmission pathway for WSSV (Liu *et al.,* 2007).

True vertical transmission (intra-ovum) of WSSV to the progeny has not been demonstrated.

In-vitro studies with primary cell cultures and *in-vivo* studies with postlarvae show that the replication cycle is approximately 20 hours at 25°C (Chang *et al.*, 1996; Chen *et al.*, 2011; Wang *et al.*, 2000).

2.3.5. Environmental factors

Disease outbreaks may be induced by stressors, such as rapid changes in salinity. Water temperature has a profound effect on disease expression, with average water temperatures of between 18 and 30°C being conducive to WSSV outbreaks (Song *et al.*, 1996; Vidal *et al.*, 2001). Under experimental challenge condition, WSSV-induced mortality in shrimp is reduced when the temperature increases above 32°C (Vidal *et al.,* 2001).

2.3.6. Geographical distribution

Infection with WSSV has been identified from crustaceans in Asia, the Mediterranean (Stentiford & Lightner, 2011), the Middle East, Oceania (Moody *et al*., 2022) and the Americas. Zones and compartments free from infection with WSSV are known within these regions (Lo *et al.,* 2012).

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No consistently effective vaccination methods have been developed for infection with WSSV.

2.4.2. Chemotherapy including blocking agents

No published or validated methods.

2.4.3. Immunostimulation

Several reports have shown that beta-glucan, vitamin C, seaweed extracts (fucoidan) and other immunostimulants may improve resistance to infection with WSSV (Chang *et al*., 2003; Chotigeat *et al*., 2004).

2.4.4. Breeding resistant strains

Progress in breeding *P. vannamei* for resistance to infections with WSSV has been reported (Cuellar-Anjel *et al.,* 2012; Huang *et al.,* 2012).

2.4.5. Inactivation methods

Method	Treatment	Reference
Heat	55°C/90 min 70°C/5 min	Chang *et al*., 1998
	50°C/60 min 60°C/1 min 70°C/0.2 min	Nakano *et al*., 1998
pH	pH 3/60 min pH 12/10 min	Chang *et al*., 1998; Balasubramanian *et al.,* 2006
UV	9.30×10^5 µWs/cm^2	Chang *et al*., 1998
Ozone	0.5 µg ml^{-1}/10 min	Chang *et al*., 1998
Chlorine	100 ppm/10 min	Chang *et al*., 1998; Balasubramanian *et al.,* 2006
Iodophore	100 ppm/10 min	Chang *et al*., 1998

2.4.6. Disinfection of eggs and larvae

For transovum transmission, disinfection of egg is likely to be effective (Lo & Kou, 1998), but this has not yet been confirmed in formal scientific trials.

2.4.7. General husbandry

Management practices in endemic areas principally involve the exclusion of WSSV from production populations or avoiding risk factors for development of clinical disease. Examples include avoiding stocking in the cold season, use of specific pathogen-free (SPF) or polymerase chain reaction (PCR)-negative seed stocks, use of biosecure water and culture systems (Withyachumnarnkul, 1999). Polyculture of shrimp and fish has been proposed to reduce WSSV transmission in infected populations (Wang *et al*., 2021).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Samples of moribund shrimp or shrimp that show clinical signs or exhibit behavioural changes (Sections 2.3) should be selected for detection of WSSV.

3.2. Selection of organs or tissues

Tissue tropism analysis from both experimentally infected shrimp and wild-captured brooders shows that tissues originating from the ectoderm and mesoderm, especially the cuticular epithelium and subcuticular connective tissues, as well as other target tissues (e.g. antennal gland, haematopoietic organ, etc.), are the main target tissues

for infection with WSSV. Samples from the pleopods, gills, haemolymph, stomach or abdominal muscle are recommended for submission (Lo *et al.*, 1997).

3.3. Samples or tissues not suitable for pathogen detection

Although WSSV infects the underlying connective tissue in the shrimp hepatopancreas and midgut, the columnar epithelial cells of these two organs are of endodermal embryonic origin (Lo *et al.*, 1997) and are not appropriate tissues for detection. The compound eye may contain a PCR inhibitor (Lo *et al.*, 1997) and is therefore not suitable for PCR-based diagnosis.

3.4. Non-lethal sampling

Gill, haemolymph or pleopod are suitable tissues for non-lethal sampling and screening by PCR.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Standard sample collection, preservation and processing methods for histological techniques can be found in Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not applicable.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore larger specimens should be processed and tested individually. Small life stages can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology					+	+	+	1				
Cell culture												
Real-time PCR	+++	+++	+++	4	+++	+++	+++	4	+++	+++	+++	4
Conventional PCR	++	++	++	2	++	++	++	2				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	2
***In-situ* hybridisation**					+	+	+	1	+	+	+	1
Bioassay					+	+	+	1				
LAMP	++	++	++	1	++	++	++	1	+	+	+	1
Ab-ELISA					+	+	+	1				
Ag-ELISA					+	+	+	1				
Other antigen detection methods					+	+	+	1				
Other methods												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test. [give definitions of abbreviations as appropriate; nPCR = nested PCR, etc. NB "RT-PCR" is reserved for reverse-transcription polymerase chain reaction methods. "real-time PCR" should always be stated in full and refers to probe-based and SYBR green assays]
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Demonstration of hypertrophied nuclei in squash preparations of the gills and/or cuticular epithelium, which may be stained or unstained.

T-E staining

A T-E staining solution may be prepared from Trypan blue 0.6%, Eosin Y 0.2%, NaCl 0.5%, phenol 0.5%, and glycerol 20% (Huang & Yu, 1995) and used as follows:

i) Place a piece of diseased tissue (e.g. a piece of gill or stomach epithelium without the cuticle) on a slide and mince with a scalpel.

ii) Add 1–2 drops of the T-E staining solution to the minced tissue, mix and allow to stain for 3–5 minutes.

iii) Lay a cover glass over the stained tissue and cover with several pieces of absorbent paper. Use a thumb to squash the mince into a single layer of cells.

If the sample was taken from a heavily infected shrimp, hypertrophied nuclei and intranuclear eosinophilic or vacuolation-like inclusion bodies can be observed using light microscopy (400–1000× magnification).

4.2. Histopathology and cytopathology

Smears

Demonstration of aggregates of WSSV virions in unstained smear preparations of haemolymph by dark-field microscopy.

NOTE: This is the simplest of the microscopic techniques and is recommended for people with limited expertise in diagnosing infection with WSSV. The aggregates appear as small reflective spots of 0.5 μm in diameter (Momoyama *et al.*, 1995).

Fixed sections

Histological changes commonly reported in susceptible species include: Hypertrophied nuclei with marginated chromatin material in virus-infected cells; eosinophilic to pale basophilic (with haematoxylin & eosin stain) stained intranuclear viral inclusions within hypertrophied nuclei and multifocal necrosis associated with pyknotic and karyorrhectic nuclei in affected tissues of ectodermal and mesodermal origin. The infection with infectious hypodermal and hematopoietic necrosis virus, another DNA virus, produces similar inclusions that need to be differentiated from those of WSSV.

4.3. Cell culture for isolation

WSSV can be isolated from primary cultures of lymphoid or ovary cells. However, it is NOT recommended to use cell culture as a routine isolation method because of: 1) the high risk of contamination, and, 2) the composition of the medium varies depending on the tissue type, host species and experimental purpose; that is, to date there is no standard or recognised medium that can be recommended. As primary cell culture is so difficult to initiate and maintain for virus isolation purposes, bioassay should be the primary means for virus propagation.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

4.4.1. Real-time PCR

The real-time PCR methods described by Durand & Lightner (2002) and Sritunyalucksana *et al.* (2006) are described here as modified and validated by Moody *et al.,* (2022).

Pathogen/ Target	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1 (Durand & Lightner, 2002[1]; GenBank Accession No. [to be included])			
WSSV ORF X	Fwd WSS1011F: TGG-TCC-CGT-CCT-CAT-CTC-AG Rev WSS1079R: GCT-GCC-TTG-CCG-GAA-ATT-A Probe: AGC-CAT-GAA-GAA-TGC-CGT-CTA-TCA-CAC-A	900 nM 900 nM 250 nM	45 cycles of: 95°C/15 sec and 60°C/1 min
Method 2 (Sritunyalucksana, 2006[1]; GenBank Accession No. [to be included])			
WSSV ORF X	Fwd CSIRO WSSV-F: CCG ACG CCA AGG GAA CT Rev CSIRO WSSV-R: TTC AGA TTC GTT ACC GTT TCC A Probe: 6FAM-CGC TTC AGC CAT GCC AGCCG-TAMRA	900 nM 900 nM 250 nM	45 cycles of: 95°C/15 sec and 60°C/1 min

[1]Method described here as modified and validated by Moody *et al.,* 2022

4.4.2. Conventional PCR

Pathogen/ Target	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1 (Lo *et al.*, 1996a; GenBank Accession No. , 1447/941 bp)			
WSSV (nested PCR)	Outer Fwd: ACT-ACT-AAC-TTC-AGC-CTA-TCTAG Rev; 146R1, 5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A	100 pmol 100 pmol	39 cycles of 94°C/1 min, 55°C/1 min, 72°C/2 min
	Inner Fwd 146F2: GTA-ACT-GCC-CCT-TCC-ATC-TCC-A Rev 146R2 TAC-GGC-AGC-TGC-TGC-ACC-TTG	100 pmol 100 pmol	39 cycles of 94°C/1 min, 55°C/1 min, 72°C/2 min

Commercial PCR kits are available. Please consult the OIE Register for kits that have been certified by the OIE (https://www.woah.org/en/what-we-offer/veterinary-products/#ui-id-5).

4.4.3. Loop-mediated isothermal amplification (LAMP) method

The protocol described here is from Kono *et al.* (2004). The LAMP method is sensitive and rapid, and it amplifies the target nucleic acids under isothermal conditions, therefore needing no sophisticated machine for thermal cycling.

DNA extraction

DNA extraction could be performed according to the protocol described in Section 4.4.2 *Conventional PCR* or by other suitable methods or by commercial kits.

LAMP reaction

i) Add DNA to a tube to set up a 25 µl reaction mixture (20 mM Tris/HCl, pH 8.8, 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 0.8M Betaine, 1.4 mM of each dNTP, 40 pmol of WSSV-FIP and -BIP primers, 5 pmol of WSSV-F3 and -B3 primers).

ii) The primer sequences are WSSV-FIP: 5'-GGG-TCG-TCG-AAT-GTT-GCC-CAT-TTT-GCC-TAC- GCA-CCA-ATC-TGT-G-3', WSSV-BIP: 5'-AAA-GGA-CAA-TCC-CTC-TCC-TGC-GTT-TTA-GAA-CGG-AAG-AAA-

CTG-CC-TT-3', WSSV-F3: ACG-GAC-GGA-GGA-CCC-AAA-TCG-A-3', WSSV-B3: 5'-GCC-TCT-GCA-ACA-TCC-TTT-CC-3'.

iii) Heat the mixture at 50°C for 5 minutes and at 95°C for 5 minutes, then chill on ice, and add 1 µl (8 U) of *Bst* DNA polymerase.

iv) Incubate the mixture at 65°C for 60 minutes, and then terminate the reaction at 80°C for 10 minutes.

v) To visualise, electrophorese 2 µl LAMP reaction products on 2% agarose gels containing ethidium bromide at a concentration of 0.5 µg ml^{-1}. This reaction produces WSSV-specific LAMP products with multiple bands of various sizes from approximately 200 bp to the loading well.

Reliable LAMP commercial kits may be an alternative for WSSV diagnosis.

4.5. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced and analysed and compared with published sequences.

4.6. *In-situ* hybridisation

Use of WSSV-specific DNA probes with histological sections is useful to demonstrate the presence of WSSV nucleic acid in infected cells (Nunan & Lightner, 1997). See Chapter 2.2.0 Section 5.5.4 for general comments on *in-situ* hybridisation.

4.7. Immunohistochemistry

See Section 4.9.

4.8. Bioassay

If SPF shrimp are available, the bioassay method based on Nunan *et al.* (1998) and Durand *et al*. (2000), is suitable for WSSV diagnosis.

4.9. Antigen detection methods

Both polyclonal and monoclonal antibodies raised against either the virus or a recombinant viral structural protein have been used in various immunological assays including western blot analysis, immunodot assay, indirect fluorescent antibody test (IFAT), immunohistochemistry (IHC) or enzyme-linked immunosorbent assay (ELISA) to detect WSSV (Huang *et al*., 1995; Poulos *et al.*, 2001; Sithigorngul *et al.*, 2006; Yoganandhan *et al.*, 2004).

4.10. Other methods

Lateral flow tests are commercially available but their performance needs to be evaluated before they can be recommended.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR is the recommended test for surveillance to demonstrate freedom of infection with WSSV in apparently healthy populations as described in Section 4.4.1.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in

endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [12]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with WSSV shall be suspected if at least one of the following criteria is met:

i) Positive result by conventional PCR

ii) Positive result by real-time PCR

iii) Positive result by LAMP method

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with WSSV is considered to be confirmed if at least one of the following criteria is met:

i) Positive results by real-time PCR and conventional PCR followed by amplicon sequencing

ii) Positive results by LAMP and conventional PCR method followed by amplicon sequencing

iii) Positive results by *in-situ* hybridisation and detection of WSSV by real-time PCR

iv) Positive results by *in-situ* hybridisation and detection of WSSV by conventional PCR followed by amplicon sequencing

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with WSSV shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs consistent with the disease as described in this chapter, with or without elevated mortality

ii) Histopathology consistent with WSSV infection

iii) Positive result by conventional PCR

iv) Positive result by real-time PCR

v) Positive result by LAMP method

vi) Positive result by *in-situ* hybridisation

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with WSSV is considered to be confirmed if at least at least one of the following criteria is met:

i) Positive results by real-time PCR and conventional PCR followed by amplicon sequencing

[12] For example transboundary commodities.

ii) Positive results by LAMP and conventional PCR method followed by amplicon sequencing

iii) Positive results by *in-situ* hybridisation and detection of WSSV by real-time PCR

iv) Positive results by *in-situ* hybridisation and detection of WSSV by conventional PCR followed by amplicon sequencing

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with WSSV are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with WSSV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR (Durand & Lightner, 2002)	Diagnosis	Clinically diseased shrimp from farms	Gill, pleopod	*Penaeus monodon*	100%	100%	Real-time PCR	Moody *et al.*, 2022
Real-time PCR (Sritunyalucksana *et al.*, 2006)	Diagnosis	Clinically diseased shrimp from farms	Gill, pleopod	*Penaeus monodon*	100%	100%	Real-time PCR	Moody *et al.*, 2022

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study,
PCR: = polymerase chain reaction.
*The nested PCR (Lo *et al.*, 1996a) is linked to false positives for WSSV when they are used to test species of *Cherax quadricarinatus* (Claydon *et al.*, 2004).

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR (Durand & Lightner, 2002)	Surveillance in apparently healthy animals	Wild populations of crustaceans	Gill, pleopod	*Penaeus merguiensis, P. esculentus, P. plebejus, Metapenaeus endeavouri, M. bennettae*	76.8%	99.7%	Bayesian latent class analysis	Moody *et al.*, 2022
Real- time PCR (Sritunyalucksana et al., 2006)	Surveillance in apparently healthy animals	Wild populations of crustaceans	Gill, pleopod	*Penaeus merguiensis, P. esculentus, P. plebejus, Metapenaeus endeavouri, M. bennettae*	82.9%	99.7%	Bayesian latent class analysis	Moody *et al.*, 2022
Two real-time PCR methods in parallel (Sritunyalucksana *et al.*, 2006 and	Surveillance in apparently healthy animals	Wild populations of crustaceans	Gill, pleopod	*Penaeus merguiensis, P. esculentus, P. plebejus, Metapenaeus*	98.3%	99.4%	Bayesian latent class analysis	Moody *et al.*, 2022

Durand & Lightner, 2002)				*endeavouri, M. bennettae*				

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study, PCR: = polymerase chain reaction.

7. References

BALASUBRAMANIAN G., SUDHAKARAN R., SYED MUSTHAQ S., SARATHI M. & SAHUL HAMEED A.S. (2006). Studies on the inactivation of white spot syndrome virus of shrimp by physical and chemical treatments, and seaweed extracts tested in marine and freshwater animal models. *J. Fish Dis.*, **29**, 569–572.

CHANG C.-F., SU M.-S., CHEN H.-Y. & LIAO I.C. (2003). Dietary β-1,3-glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. *Fish Shellfish Immunol.*, **15**, 297–310.

CHANG P.S., CHEN H.C. & WANG Y.C. (1998). Detection of white spot syndrome associated baculovirus in experimentally infected wild shrimp, crab and lobsters by *in situ* hybridization*. Aquaculture*, **164**, 233–242.

CHANG P.S., LO C.F., WANG Y.C. & KOU G.H. (1996). Identification of white spot syndrome associated baculovirus (WSBV) target organs in the shrimp *Penaeus monodon* by *in situ* hybridization. *Dis. Aquat. Org.,* **27**, 131–139.

CHANG Y., CHEN T., LIU W., HWANG J.& LO C. (2011). Assessment of the roles of copepod *Apocyclops royi* and bivalve mollusk *Meretrix lusoria* in white spot syndrome virus transmission. *Mar. Biotechnol*., **13**, 909–917.

CHEN I.T, AOKI T., HUANG Y.T., HIRONO I., CHEN T.C., HUANG J.Y., CHANG G.D., LO C.F., WANG H.C. (2011). White spot syndrome virus induces metabolic changes resembling the Warburg effect in shrimp hemocytes in the early stage of infection. *J. Virol.,* **85**, 12919–12928.

CHEN W.Y., ZHANG H., GU L., LI F. & YANG F. (2012). Effects of high salinity, high temperature and pH on capsid structure of white spot syndrome virus. *Dis. Aquat. Org.,***101**, 167–171.

CHOTIGEAT W., TONGSUPA S., SUPAMATAYA K. & PHONGDARA A. (2004). Effect of fucoidan on disease resistance of black tiger shrimp. *Aquaculture*, **233**, 23–30.

CLAYDON K., CULLEN B. & OWENS L. (2004). OIE white spot syndrome virus PCR gives false-positive results in *Cherax quadricarinatus. Dis. Aquat. Org.,* **62**, 265–268.

CORBEL V., ZUPRIZAL Z., SHI C., HUANG, SUMARTONO, ARCIER J.-M. & BONAMI J.-R. (2001). Experimental infection of European crustaceans with white spot syndrome virus (WSSV). *J. Fish Dis*., **24**, 377–382.

CUELLAR-ANJEL J., WHITE-NOBLE B., SCHOFIELD P., CHAMORRO R. & LIGHTNER D.V. (2012). Report of significant WSSV-resistance in the Pacific white shrimp, *Litopenaeus vannamei*, from a Panamanian breeding program. *Aquaculture*, **368–369**, 36–39.

DESRINA, PRAYITNO S.B, VERDEGEM M.C.J, VERRETH J.A.J. & VLAK J.M. (2022). White spot syndrome virus host range and impact on transmission. *Rev. Aquacult*., 1–18.

DESRINA, VERRETH J.A.J., PRAYITNO S.B., ROMBOUT J.H.W.M., VLAK J.M. & VERDEGEM M.C.J. (2013). Replication of white spot syndrome virus (WSSV) in the polychaete *Dendronereis* spp. *J. Invertebr. Pathol*., **114**, 7–10.

DURAND S.V. & LIGHTNER D.V. (2002). Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. *J. Fish Dis*., **25**, 381–389.

DURAND S.V., TANG K.F.J. & LIGHTNER D.V. (2000). Frozen commodity shrimp: potential avenue for introduction of white spot syndrome virus and yellow head virus. *J. Aquat. Anim. Health,* **12**, 128–135.

EAST I.J. (2008). Addressing the problems of using the polymerase chain reaction technique as a stand-alone test for detecting pathogens in aquatic animals. *Sci. Tech. Rev*., **27**, 829–837.

ESCOBEDO-BONILLA C. M., ALDAY-SANZ V., WILLE M., SORGELOOS P., PENSAERT M.B. & NAUWYNCK H.J. (2008). A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. *J. Fish Dis.*, **31**, 1–18.

FLEGEL T.W. (1997). Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. *World J. Microbiol. Biotechnol.,* **13**, 433–442.

HASSON K.W., FAN Y., REISINGER T., VENUTI J. & VARNER P.W. (2006). White-spot syndrome virus (WSSV) introduction into the Gulf of Mexico and Texas fresh water systems through imported, frozen bait-shrimp. *Dis. Aquat. Org.*, **71**, 91–100.

HARYADI D., VERRETH J.A.J., VERDEGEM M.C.J. & VLAK J.M. (2015). Transmission of white spot syndrome virus (WSSV) from *Dendronereis* spp. (Peters) (Nereididae) to penaeid shrimp. *J. Fish Dis.*, **38**, 419-428.

HE J. & ZHOU H. (1996). Infection route and host species of white spot syndrome baculovirus. *Acta Sci. Natur. Univ. Sunyatseni*, **38**, 65–69.

HEIDARIEH M., SOLTANI M., MOTAMEDI SEDEH F. & SHEIKHZADEH N. (2013). Low water temperature retards white spot syndrome virus replication in *Astacus leptodactylus* Crayfish. *Acta Sci. Vet.*, **41,** 1–6.

HUANG J. & YU J. (1995). A new staining method for on-site observation of viral inclusion bodies of penaeid shrimp. (*Chinese J.*). *Mar. Fish. Res.*, **16**, 31–39.

HUANG J., YU J., WANG X.-H., SONG X.-L., MA C.-S., ZHAO F.-Z. & YANG C.-H. (1995). Survey on the pathogen and route of transmission of baculoviral hypodermal and hematopoietic necrosis in shrimp by ELISA of monoclone antibody. (*Chinese J.*). *Mar. Fish. Res.*, **16**, 40–50.

HUANG Y., YIN Z., WENG S., HE J. & LI S. (2012). Selective breeding and preliminary commercial performance of *Penaeus vannamei* for resistance to white spot syndrome virus (WSSV). *Aquaculture*, **364–365**, 111–117.

KONO T., SAVAN R., SAKAI M., & ITAMI T. (2004). Detection of white spot syndrome virus in shrimp by loop-mediated isothermal amplification. *J. Virol. Methods*, **115**, 59–65.

LEI Z.-W., HUANG J., SHI C.-Y., ZHANG L.-J. & YU K.-K. (2002). Investigation into the hosts of white spot syndrome virus (WSSV). *Oceanol. Limnol. Sin.*, **33**, 250–258.

LI Q., ZHANG J.H., CHEN Y.J. & YANG F. (2004). White spot syndrome virus (WSSV) infectivity for *Artemia* at different developmental stages. *Dis. Aquat. Org.*, **57**, 261–264.

LIGHTNER D.V. (1996). A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. Baton Rouge, Louisiana, USA: World Aquaculture Society, 1996.

LIU B., YU Z.M., SONG X.X. & GUAN Y.Q. (2007). Studies on the transmission of WSSV (white spot syndrome virus) in juvenile *Marsupenaeus japonicus* via marine microalgae. *J. Invertebr. Pathol.*, **95**, 87–92.

LO C.F., AOKI T., BONAMI J.R., FLEGEL T.W., LEU J.H., LIGHTNER D.V., STENTIFORD G., SÖDERHÄLL K., WALKER P.W. WANG H.C.., XUN X., YANG F. & VLAK J.M. (2012). *Nimaviridae*. *In:* Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses, King A.M.Q., Adams M.J., Carstens E.B., & Lefkowitz E.J., eds. Elsevier Academic Press, San Diego, CA. USA, pp 229–234.

LO C.F., HO C.H., CHEN C.H., LIU K.F., CHIU Y.L., YEH P.Y., PENG S.E., HSU H.C., LIU H.C., CHANG C.F., SU M.S., WANG C.H. & KOU G.H. (1997). Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *Dis. Aquat. Org.,* **30**, 53–72.

LO C.F., HO C.H., PENG S.E., CHEN C.H., HSU H.C., CHIU Y.L., CHANG C.F., LIU K.F., SU M.S., WANG C.H. & KOU G.H. (1996b). White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. *Dis. Aquat. Org.*, **27**, 215–225.

LO C.F. & KOU G.H. (1998). Virus-associated white spot syndrome of shrimp in Taiwan: a review. *Fish Pathol.,* **33**, 365–371.

LO C.F., LEU J.H., HO C.H., CHEN C.H., PENG S.E., CHEN Y.T., CHOU C.M., YEH P.Y., HUANG C.J., CHOU H.Y., WANG C.H. & KOU G.H. (1996a). Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Dis. Aquat .Org.*, **25**, 133–141.

MAEDA M., ITAMI T., MIZUKI E., TANAKA R., YOSHIZU Y., DOI K., YASUNAGA-AOKI C., TAKAHASHI Y. & KAWARABATA T. (2000). Red swamp crawfish (*Procambarus clarkii*): an alternative experimental host in the study of white spot syndrome virus. *Acta Virol.,* **44**, 371–374.

MCCOLL K.A., SLATER J., JEYASEKARAN G., HYATT A.D. & CRANE M.ST.J. (2004). Detection of White Spot Syndrome virus and Yellow head virus in prawns imported into Australia. *Australian Vet. J.*, **82**, 69–74.

MOMOYAMA K., HIRAOKA M., INOUYE K., KIMURA T. & NAKANO H. (1995). Diagnostic techniques of the rod-shaped nuclear virus infection in the kuruma shrimp, *Penaeus japonicus. Fish Pathol.,* **30**, 263–269.

MOMOYAMA K., HIRAOKA M., NAKANO H., KOUBE H., INOUYE K. & OSEKO N. (1994). Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus*, in Japan in 1993: Histopathological study. *Fish Pathol.,* **29**, 141–148.

MOMOYAMA K., HIRAOKA M., NAKANO H. & SAMESHIMA M. (1998). Cryopreservation of penaeid rod-shaped DNA virus (PRDV) and its survival in sea water at different temperatures. *Fish Pathol.,* **33**, 95–96.

MOODY N.J.G., MOHR P.G., WILLIAMS L.M., CUMMINS D.M., HOAD J., SLATER J., VALDETER S.T., COLLING A., SINGANALLUR N.B., GARDNER I.A., GUDKOVS N. & CRANE M.ST.J. (2022). Performance characteristics of two real-time, TaqMan polymerase chain reaction assays for the detection of WSSV in clinically diseased and apparently health prawns. *Dis. Aquat. Org.*, https://www.int-res.com/prepress/d03687.html.

NAKANO H., HIRAOKA M., SAMESHIMA M., KIMURA T. & MOMOYAMA K. (1998). Inactivation of penaeid rod-shaped DNA virus (PRDV), the causative agent of penaeid acute viraemia (PAV), by chemical and physical treatments. *Fish Pathol.,* **33**, 65–71.

NUNAN L.M. & LIGHTNER D.V. (1997). Development of a non-radioactive gene probe by PCR for detection of white spot syndrome virus (WSSV). *J. Virol. Methods,* **63**, 193–201.

NUNAN L.M. & LIGHTNER D.V. (2011). Optimized PCR assay for detection of white spot syndrome virus (WSSV). *J. Virol. Methods,* **171**, 318–321.

NUNAN L.M., POULOS B.T. & LIGHTNER D.V. (1998). The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. *Aquaculture,* **160**, 19–30.

POULOS B.T., PANTOJA C.R., BRADLEY-DUNLOP D., AGUILAR J. & LIGHTNER D.V. (2001). Development and application of monoclonal antibodies for the detection of white spot syndrome virus of penaeid shrimp. *Dis. Aquat. Org.,* **47**, 13–23.

SAHUL HAMEED A.S., ANILKUMAR M., STEPHEN RAJ M.L. & JAYARAMAN K. (1998). Studies on the pathogenicity of systemic ectodermal and mesodermal baculovirus and its detection in shrimp by immunological methods. *Aquaculture*, **160**, 31–45.

SAHUL HAMEED A.S., YOGANANDHAN K., SATHISH S., RASHEED M., MURUGAN V. & JAYARAMAN K. (2001). White spot syndrome virus (WSSV) in two species of freshwater crabs (*Paratelphusa hydrodomous* and *P. pulvinata*). *Aquaculture*, **201**, 179–186.

SANCHEZ-PAZ A. (2010). White spot syndrome virus: an overview on an emergent concern. *Vet. Res.*, **41**, 43.

SATHEESH KUMAR S., ANANDA BHARATHI R., RAJAN J.J.S., ALAVANDI S.V., POORNIMA M., BALASUBRAMANIAN C.P. & PONNIAH A.G. (2013). Viability of white spot syndrome virus (WSSV) in sediment during sun-drying (drainable pond) and under non-drainable pond conditions indicated by infectivity to shrimp. *Aquaculture*, **402–403**, 119–126.

SITHIGORNGUL W., RUKPRATANPORN S., PECHARABURANIN N., LONGYANT S., CHAIVISUTHANGKURA P. & SITHIGORNGUL P. (2006). A simple and rapid immunochromatographic test strip for detection of white spot syndrome virus (WSSV) of shrimp. *Dis. Aquat. Org.,* **72**, 101–106.

SONG X., HUANG J., WANG C., YU J., CHEN B. & YANG C. (1996). Artificial infection of brood shrimp of *Penaeus chinensis* with hypodermal and hematopoietic necrosis baculovirus. *J. Fish. China,* **20**, 374–378.

SRITUNYALUCKSANA K., SRISALA J., MCCOLL K., NIELSEN L. & FLEGEL T.W. (2006). Comparison of PCR methods for white spot syndrome virus (WSSV) infections in penaeid shrimp. *Aquaculture,* 255, 95–104.

STENTIFORD G.D., BONAMI J.R. & ALDAY-SANZ V. (2009). A critical review of susceptibility of crustaceans to Taura Syndrome, yellowhead disease and white spot disease and implications of inclusion of these diseases in European legislation. *Aquaculture,* **291**, 1–17.

STENTIFORD G.D. & LIGHTNER D.V. (2011). Cases of white spot disease (WSD) in European shrimp farms. *Aquaculture,* **319**, 302–306.

TSAI M.F., KOU G.H., LIU H.C., LIU K.F., CHANG C.F., PENG S.E., HSU H.C., WANG C.H. & LO C.F. (1999). Long-term presence of white spot syndrome virus (WSSV) in a cultivated shrimp population without disease outbreaks. *Dis. Aquat. Org.,* **38**, 107–114.

VANPATTEN K.A., NUNAN L.M. & LIGHTNER D.V. (2004). Sea birds as potential vectors of penaeid shrimp viruses and the development of a surrogate laboratory model utilizing domestic chickens. *Aquaculture*, **241**, 31–46.

VENEGAS C.A., NONAKA L., MUSHIAKE K., SHIMIZU K., NISHIZAWA T. & MUROGA K. (1999). Pathogenicity of penaeid rod-shaped DNA virus (PRDV) to kuruma prawn in different developmental stages. *Fish Pathol.,* **34**, 19–23.

VIDAL O.M., GRANJA C.B., ARANGUREN F., BROCK J.A. & SALAZAR M. (2001). A profound effect of hyperthermia on survival of *Litopenaeus vannamei* juveniles infected with white spot syndrome virus. *J. World Aquac. Soc.,* **32**, 364–372.

VIJAYAN K.K., STALIN RAJ V., BALASUBRAMANIAN C.P., ALAVANDI S.V., THILLAI SEKHAR V. & SANTIAGO T.C. (2005). Polychaete worms – a vector for white spot syndrome virus (WSSV). *Dis. Aquat. Org.,* **63**, 107–111.

WANG C.H., YANG H.N., TANG C.Y., LU C.H., KOU G.H. & LO C.F. (2000). Ultrastructure of white spot syndrome virus development in primary lymphoid organ cell cultures. *Dis. Aquat. Org.*, **41**, 91–104.

WANG H.C., HIRONO I, MANINGAS M.B.B., SOMBOONWIWA K., STENTIFORD G. & ICTV REPORT CONSORTIUM. (2019). ICTV Virus Taxonomy Profile: *Nimaviridae*. *In:* Virus Taxonomy: The ICTV 10th Report on Virus Classification and Taxon Nomenclature. The ICTV website (www.ictv.global/report/nimaviridae).

WANG M., CHEN Y., ZHAO Z., WENG S., YANG J., LIU S., LIU C., YUAN F., AI B., ZHANG H., ZHANG M., LU L., YUAN K., YU Z., MO B., LIU X., GAI C., LI Y., LU R., ZHONG Z., ZHENG L., FENG G., LI S.C. & HE J. (2021). A convenient polyculture system that controls a shrimp viral disease with a high transmission rate. *Commun Biol*., **4**, 1276.

WITHYACHUMNARNKUL B. (1999). Results from black tiger shrimp *Penaeus monodon* culture ponds stocked with postlarvae PCR-positive or -negative for white-spot syndrome virus (WSSV). *Dis. Aquat. Org.,* **39**, 21–27.

WONGTEERASUPAYA C., VICKERS J.E., SRIURAIRATANA S., NASH G.L., AKARAJAMORN A., BOONSAENG V., PANYIM S., TASSANAKAJON A., WITHYACHUMNARNKUL B. & FLEGEL T.W. (1995). A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn *Penaeus monodon*. *Dis. Aquat. Org.,* **21**, 69–77.

WU J.L., SUZUKI K., ARIMOTO M., NISHIZAWA T. & MUROGA K. (2002). Preparation of an Inoculum of White Spot Syndrome Virus for Challenge Tests in Penaeus japonicus. *Fish Pathol.,* **37**, 65–69.

WU W., WU B., YE T., HUANG H., DAI C., YUAN J. & WANG W. (2013). TCTP is a critical factor in shrimp immune response to virus infection. *PloS One*, *8*, e74460.

YAN D.C., DONG S.L., HUANG J., YU X.M., FENG M.Y. & LIU X.Y. (2004). White spot syndrome virus (WSSV) detected by PCR in rotifers and rotifer resting eggs from shrimp pond sediments. *Dis. Aquat. Org.,* **59**, 69–73.

YAN D.C., DONG S.L., HUANG J.& ZHANG J.S. (2007). White spot syndrome virus (WSSV) transmission from rotifer inoculum to crayfish. *J. Invertebr. Pathol*., **94**, 144–148.

YOGANANDHAN K., SYED MUSTHAQ S., NARAYANAN R.B. & SAHUL HAMEED A.S. (2004). Production of polyclonal antiserum against recombinant VP28 protein and its application for the detection of white spot syndrome virus in crustaceans. *J. Fish Dis.,* **27**, 517–522.

ZENG Y. (2021). Molecular epidemiology of white spot syndrome virus in the world. *Aquaculture*, **537**, 736509. https://doi.org/10.1016/j.aquaculture.2021.736509.

ZHAN W.B., WANG Y.H., FRYER J.L., YU K.K., FUKUDA H. & MENG Q.X. (1998). White Spot Syndrome Virus Infection of Cultured Shrimp in China. *J. Aquat. Anim. Health*, **10**, 405–410.

ZHANG J.S., DONG S.L. DONG Y.W., TIAN X.L., CAO Y.C. & LI Z.J., YAN D.C. (2010). Assessment of the role of brine shrimp *Artemia* in white spot syndrome virus (WSSV) transmission. *Vet. Res. Commun*., **34**, 25–32.

ZHANG J.S., DONG S.L., DONG Y.W., TIAN X.L. & HOU C.Q. (2008). Bioassay evidence for the transmission of WSSV by the harpacticoid copepod *Nitocra* sp. *J. Invertebrate Pathol*., **97**, 33–39.

ZHANG J.S., DONG S.L., TIAN X.L., DONG Y.W., LIU X.Y. & YAN D.C. (2006). Studies on the rotifer (*Brachionus urceus* Linnaeus, 1758) as a vector in white spot syndrome virus (WSSV) transmission. *Aquaculture*, **261**, 1181–1185.

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NB: There are OIE Reference Laboratories for infection with white spot syndrome virus
(please consult the OIE web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3http://www.oie.int/).
Please contact the OIE Reference Laboratories for any further information on
infection with white spot syndrome virus

NB: FIRST ADOPTED IN 1997 AS WHITE SPOT DISEASE. MOST RECENT UPDATES ADOPTED IN 2018.

CHAPTER 2.3.1.

INFECTION WITH APHANOMYCES INVADANS (EPIZOOTIC ULCERATIVE SYNDROME)

1. Scope

Infection with *Aphanomyces invadans* means all infections caused by the oomycete ~~fungus~~ *A. invadans* of the Genus *Aphanomyces* and Family *Leptolegniaceae.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Infection with *A. invadans* is most commonly known as epizootic ulcerative syndrome (EUS) ~~(Food and Agriculture Organization of the United Nations [FAO], 1986).~~ It has also been known as red spot disease (RSD) (Mckenzie & Hall, 1976), mycotic granulomatosis (MG) (Egusa & Masuda, 1971; Hanjavanit, 1997) and ulcerative mycosis (UM) (Noga & Dykstra, 1986). The disease is caused by the oomycete *Aphanomyces invadans*.

Infection with *A. invadans* is a seasonal epizootic condition of great importance in wild and farmed freshwater and estuarine fish. It is clinically characterised by the presence of invasive, non-septate hyphae in skeletal muscle, usually leading to a granulomatous response. Infections with *A. invadans* have spread widely since the first outbreak in 1971 in Japan and to date a high degree of genetic homogeneity is observed for this species based on publicly available genome sequences (Dieguez-Uribeondo *et al.,* 2009; European Food Safety Authority [EFSA] 2011a; Huchzermeyer *et al.,* 2018; Iberahim *et al.,* 2018; Lilley *et al.,* 2003). Other pathogenic viruses (mostly rhabdoviruses), bacteria (mainly *Aeromonas hydrophila*), fungi, oomycetes and parasites are routinely co-isolated from *A. invadans*-infected fish (Iberahim *et al.,* 2018).

Aphanomyces invadans is within a group of organisms commonly known as the water moulds. Although long-regarded as fungi because of their characteristic filamentous growth, this group, the Oomycota, is not considered a member of the Eumycota (true fungi) but is classified with diatoms and brown algae in a group called the Heterokonta or Stramenopiles within the Kingdom Chromista (Cavalier-Smith & Chao 2006; Tsui et al., 2009). Junior synonyms of *A. invadans* include *Aphanomyces piscicida* and *Aphanomyces invaderis*.

2.1.2. Survival and stability in processed or stored samples

There is limited published data on the stability of the pathogen in host tissues. It is not clear whether the pathogen continues to grow for some time following the death of the host (Oidtmann, 2012).

Aphanomyces invadans cultures can be maintained for extended periods in glucose phosphate broth (6 weeks at 10°C), agar slopes and sodium phosphate buffer (over 6 months at 20°C) (Lilley *et al.,* 1998).

2.1.3. Survival and stability outside the host

How *A. invadans* survives outside the host is unclear (Oidtmann, 2012). It is assumed that the motile zoospores, which are released from an infected fish, will encyst when unsuccessful in finding a suitable substrate to grow on (Oidtmann, 2012). Encysted zoospores of *A. invadans* are capable of releasing a new zoospore generation instead of germinating in a process called repeated zoospore emergence (Dieguez-Uribeondo *et al.,* 2009). There is no suitable method to recover or isolate the encysted zoospore from affected fish ponds (Afzali *et al.,* 2013). How long the encysted spore can survive in water or on a non-fish substrate is unclear. In an *in-vitro* experiment, the encysted zoospore survived for at least 19 days (Lilley *et al.,* 2001).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Table 2.1. Fish species susceptible to infection with **Aphanomyces invadans**

Family	Scientific name	Common name
Alestidae	*Brycinus lateralis*	striped robber
	Hydrocynus vittatus	tigerfish
	Micralestes acutidens	silver robber
Ambassidae	*Ambassis agassizii*	chanda perch
Apogonidae	*Glossamia aprion*	mouth almighty
Ariidae	*Arius sp.*	fork-tailed catfish
Belonidae	*Strongylura kreffti*	long tom
Centrarchidae	*Lepomis macrochirus*	bluegill
	Micropterus salmoides	largemouth black bass
Channidae	*Channa marulius*	great snakehead fish
	Channa striatus	striped snakehead
Cichlidae	*Coptodon rendalli*	redbreast tilapia
	Oreochromis andersoni	three-spoted tilapia
	Oreochromis machrochir	greenhead tilapia
	Sargochromis carlottae	rainbow bream
	Sargochromis codringtonii	green bream
	Sargochromis giardi	pink bream
	Serranochromis angusticeps	thinface largemouth
	Serranochromis robustus	Nembwe
	Tilapia sparrmanii	banded tilapia
Clariidae	*Clarias gariepinus*	sharptooth African catfish
	Clarias ngamensis	blunt-toothed African catfish
	Clarius batrachus	walking catfish
Clupeidae	*Alosa sapidissima*	American shad
	Brevoortia tyrannus	Atlantic menhaden
	Nematalosa erebi	bony bream
Cyprinidae	*Barbus paludinosus*	straightfin barb
	Barbus poechii	dashtail barb
	Barbus thamalakanensis	Thamalakane barb
	Barbus unitaeniatus	longbeard barb
	Carassius auratus	goldfish
	Catla catla	catla
	Cirrhinus mrigala	mrigal
	Esomus sp.	flying barb
	Labeo cylindricus	red-eye labeo
	Labeo lunatus	upper Zambezi labeo
	Labeo rohita	rohu
	Puntius gonionotus	silver barb
	Puntius sophore	pool barb
	Rohtee sp.	keti-Bangladeshi
Eleotridae	*Oxyeleotris lineolatus*	sleepy cod
	Oxyeleotris marmoratus	marble goby

Family	Scientific name	Common name
Gobiidae	*Glossogobius giuris*	bar-eyed goby
	Glossogobius sp.	goby
	Tridentiger obscures obscures	dusky tripletooth goby
Helostomatidae	*Helostoma temmincki*	kissing gourami
Hepsetidae	*Hepsetus odoe*	African pike
Ictaluridae	*Ameiurus melas*	black bullhead
	Ameiurus nebulosus	black bullhead
	Amniataba percoides	striped grunter
	Ictalurus punctatus	channel catfish
Kurtidae	*Kurtus gulliveri*	nursery fish
Latidae	*Lates calcarifer*	barramundi or sea bass
Lutjanidae	*Lutjanus argentimaculatus*	mangrove jack
Melanotaeniidae	*Melanotaenia splendida*	rainbow fish
Mormyridae	*Marcusenius macrolepidotus*	bulldog
	Petrocephalus catostoma	churchill
Mugilidae	*Mugilidae (Mugil spp.; Liza spp.)*	mullets
	Mugil cephalus	grey mullet or striped mullet
	Mugil curema	white mullet
	Myxus petardi	mullet
Osmeroidei	*Plecoglossus altivelis*	ayu
Osphronemidae	*Colisa lalia*	dwarf gourami
	Osphronemus goramy	giant gourami
	Trichogaster pectoralis	snakeskin gourami
	Trichogaster trichopterus	three-spot gourami
Osteoglossidae	*Scleropages jardini*	saratoga
Percichthyidae	*Maccullochella ikei*	freshwater cod
	Maccullochella peelii	Murray cod
	Macquaria ambigua	golden perch
	Macquaria novemaculeata	Australian bass
Platycephalidae	*Platycephalus fuscus*	dusky flathead
Psettodidae	*Psettodes sp.*	spiny turbot
Salmonidae	*Oncorhynchus mykiss*	rainbow trout
Scatophagidae	*Scatophagus argus*	spotted scat
	Selenotoca multifasciata	striped scat
Schilbeidae	*Schilbe intermedius*	silver catfish
	Schilbe mystus	African butter catfish
Sciaenidae	*Bairdiella chrysoura*	drums or croakers
	Pogonias cromis	black drum
Sillaginae	*Sillago ciliata*	sand whiting
Siluridae	*Silurus glanis*	wels catfish
Soleidae	*Aseraggodes macleayanus*	narrow banded sole
Sparidae	*Acanthopagrus australis*	yellowfin sea bream
	Acanthopagrus berda	black bream
	Archosargus probatocephalus	sheepshead
Synbranchidae	*Fluta alba*	swamp eel
Terapontidae	*Anabas testudineus*	climbing perch
	Bidyanus bidyanus	silver perch
	Leiopotherapon unicolor	spangled perch
	Scortum barcoo	Barcoo Grunter
	Therapon sp.	therapon
Toxotidae	*Toxotes chatareus*	common archerfish
	Toxotes lorentzi	primitive acherfish

2.2.2. Species with incomplete evidence for susceptibility [under study]

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *A. invadans* according to Chapter 1.5 of the *Aquatic Code* are: [under study]

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Subadult and adult fish are usually described as the susceptible life stages to natural outbreaks of EUS (FAO, 2009). However, there are reports of infection with *A. invadans* being found in early life stages (fish fry or fish larvae) (Baldock et al., 2005; EFSA 2011a). While the size of the fish does not determine an EUS outbreak (Cruz-Lacierda & Shariff, 1995), younger fish seem to be more prone to EUS compared with adult fish (Gomo *et al.,* 2016; Pagrut *et al.,* 2017).

An experimental injection of *A. invadans* into the yearling life stage of ~~Indian major carp,~~ catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*), revealed resistance to *A. invadans* (Pradhan *et al.,* 2007), even though they are naturally susceptible species. Experimental infections demonstrated that goldfish (*Carassius auratus*) are susceptible (Hatai *et al.,* 1977; 1994), but common carp (*Cyprinus carpio*) (Wada *et al.,* 1996), Nile tilapia (*Oreochromis niloticus*) (Khan *et al.,* 1998) and European eel (*Anguilla anguilla*), (Oidtmann *et al.,* 2008) are considered resistant.

2.2.4. Distribution of the pathogen in the host

During the course of an infection with *A. invadans*, the free-swimming zoospore attaches to the skin of a fish host, encysts and germinates to develop hyphae invading and ramifying through host tissues (Kiryu *et al.,* 2003; Lilley *et al.,* 1998). The hyphal invasion and associated pathology are not confined to the region of dermal ulcers. The hyphae readily invade the body cavity and produce mycotic granulomas in all the visceral organs (Vishwanath *et al.,* 1998). In fish either suspected or confirmed to be infected with *A.~~phanomyces~~ invadans,* hyphae have also been observed in kidney, liver, spleen, pancreatic tissue, gut, parietal peritoneum, swim bladder, gonads, spinal cord, meninges, vertebrae, inter-muscular bones, the mouth region, and the orbits (Chinabut & Roberts, 1999; Vishwanath *et al.,* 1998; Wada *et al.,* 1996).

2.2.5. Aquatic animal reservoirs of infection

~~There is no information to indicate that fish can be lifelong carriers of *A. invadans.*~~ Generally, most infected fish die during an outbreak. Although some fish with mild or moderate infections could recover, they are unlikely to be lifelong carriers of *A. invadans*.

2.2.6. Vectors

No data available.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

The prevalence of infection with *A. invadans* in the wild and in aquaculture farms may be high (20–90%), in endemic areas with high levels of mortality observed. Mortality patterns appear to be seasonal and can vary substantially (Herbert *et al.,* 2019).

2.3.2. Clinical signs, including behavioural changes

Fish usually develop red spots or small-to-large ulcerative lesions on the body. The occurrence of skin lesions and ultimately mortality varies according to fish species. Fish presenting with lesions are usually weak, appear darker in colour, have a reduced appetite, are immobile and may float at the surface of the water. Generally infected fish are encountered in shallow water and present a retarded ability to escape capture occasionally followed by short lived bouts of hyperactivity characterised by jerky movements (Huchzermeyer *et al.,* 2018; Iberahim *et al.,* 2018).

2.3.3 Gross pathology

Early-stage lesions or mildly infected fish are characterised by red spots observed on the lateral body surface, head, operculum or caudal peduncle of the infected fish. Scales of infected fish are often protruding or lost. In

severe cases, swollen haemorrhagic areas, massive inflammation and large deep ulcers exposing the underlying necrotic muscle tissue are observed (Huchzermeyer *et al.,* 2018; Iberahim *et al.,* 2018). In advanced stages of the disease, the severity of the disease results in death of the fish (Hawke *et al.,* 2003; Iberahim *et al.,* 2018).

2.3.4. Modes of transmission and life cycle

Aphanomyces invadans has an aseptate fungal-like mycelia structure. This oomycete has two typical zoospore forms. The primary zoospore consists of round cells that develop inside the sporangium. The primary zoospore is released to the tip of the sporangium where it forms a spore cluster. It quickly transforms into the secondary zoospore, which is a reniform, laterally biflagellate cell and can swim freely in the water. The secondary zoospore remains motile for a period that depends on the environmental conditions and presence of the fish host or substratum. Typically, the zoospore encysts and germinates to produce new hyphae, although further tertiary generations of zoospores may be released from cysts (repeated zoospore emergence or polyplanetism) (Lilley *et al.,* 1998). The *A. invadans* zoospores can be horizontally transmitted from one fish to another through the water supply. It is believed that only the secondary zoospores or free-swimming stage zoospores are capable of attaching to the damaged skin of fish and germinating into hyphae. If the secondary zoospores cannot find the susceptible species or encounter unfavourable conditions, they can encyst in the pond environment. The cysts may wait for conditions that favour their transformation into tertiary generations of zoospores that are also in the free-swimming stage. The encysting property of *A. invadans* may play an important role in the cycle of outbreaks in endemic areas.

2.3.5. Environmental factors

Under natural conditions, infection with *A. invadans* has been reported at water temperatures in the range 10–33°C (Bondad-Reantaso *et al.,* 1992; Hawke *et al.,* 2003) often associated with massive rainfall (Bondad-Reantaso *et al.,* 1992). These conditions favour sporulation of *A. invadans* (Lumanlan-Mayo *et al.*, 1997), and temperatures of 17–19°C have been shown to delay the inflammatory response of fish to oomycete infection (Catap & Munday, 1998, Chinabut *et al.*, 1995). In some countries, outbreaks occur in wild fish first and then spread to fish ponds. Normally, a bath infection of *A. invadans* in healthy susceptible fish species does not result in clinical signs of disease. The presence of other pathogens (viruses, bacteria or ectoparasites, skin damage, water temperature (between 18 and 22 °C), low pH (6.0–7.0) and low oxygen concentration in the water have all been hypothesised as predisposing factors for infection or factors influencing the expression of the disease (Oidtmann, 2012; Iberahim *et al.,* 2018).

Movements of live ornamental fish from countries from which infection with *A. invadans* is confirmed may spread the disease as was the case with the outbreak in Sri Lanka (Balasuriya, 1994). Flooding also caused the spread of infection with *A. invadans* in Bangladesh and Pakistan (Lilley *et al.,* 1998). Once an outbreak occurs in rivers/canals, the disease can spread downstream as well as upstream where the susceptible fish species exist.

Aphanomyces invadans grows best at 20–30°C; it does not grow *in-vitro* at 37°C. Water salinity over 2 parts per thousand (ppt) can stop spread of the agent. Under laboratory conditions the optimal growth temperature range for *A. invadans* is 19–22°C, while under natural conditions *A. invadans* seems to be more robust (Hawke *et al.,* 2003).

2.3.6. Geographical distribution

Infection with *A. invadans* was first reported in farmed freshwater ayu (*Plecoglossus altivelis*) in Asia in 1971 (Egusa & Masuda, 1971). It was later reported in estuarine fish, particularly grey mullet (*Mugil cephalus*) in eastern Australia in 1972 (Fraser *et al.*, 1992; Mckenzie & Hall, 1976). Infection with *A. invadans* has extended its range into South-East and South Asia, and into West Asia (Lilley *et al.*, 1998; Tonguthai, 1985). Outbreaks of ulcerative disease in menhaden (*Brevoortia tyrannus*) in North America had the same aetiological agent as the disease observed in Asia (Blazer *et al.*, 1999; Lilley *et al.*, 1997a; Vandersea *et al.*, 2006). The first confirmed outbreaks of infection with *A. invadans* on the African continent occurred in 2007, and were connected to the Zambezi-Chobe river system (Andrew *et al.,* 2008; FAO, 2009; Huchzermeyer & Van der Waal, 2012; McHugh *et al.,* 2014). In 2010 and 2011, infection with *A. invadans* appeared in wild freshwater fish in Southern Africa and in wild brown bullhead fish in North America. Infection with *A. invadans* has been reported from more than 20 countries in four continents: North America, Southern Africa, Asia and Australia.

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

There is no protective vaccine available.

2.4.2. Chemotherapy including blocking agents

There is no effective treatment for *A. invadans*-infected fish in the wild and in aquaculture ponds.

2.4.3. Immunostimulation

Experimentally infected snakehead fish fed a vitamin-supplemented feed exhibited clinical signs of infection with *A. invadans* but had higher survival than controls (Miles *et al*., 2001).

2.4.4. Breeding resistant strains

No data available.

2.4.5. Inactivation methods

To minimise fish losses in infected fish ponds water exchange should be stopped and lime or hydrated lime and/or salt should be applied (Lilley *et al.,* 1998). Preparing fish ponds by sun-drying and liming are effective disinfection methods for *A. invadans* (EFSA 2011b; Kumar *et al.,* 2020; Oidtmann, 2012). Similar to other oomycetes or water moulds, general disinfection chemicals effectively destroy *A. invadans* that might contaminate farms, fish ponds or fishing gear (Iberahim *et al.,* 2018).

2.4.6. Disinfection of eggs and larvae

Routine disinfection of fish eggs and larvae against water moulds is effective against *A. invadans*. It should be noted that there is no report of the presence of *A. invadans* in fish eggs or larvae.

2.4.7. General husbandry

Control of *A. invadans* in natural waters is probably impossible. In outbreaks occurring in small, closed water bodies or fish ponds, treating water with agricultural limes and improving water quality, together with removal of infected fish, is often effective in reducing mortalities and controlling the disease. Preventing entry of water from *A. invadans*-infected water bodies into fish ponds can prevents spread of the disease into farms. Sodium chloride or salt and agricultural lime are safe and effective chemicals for treating or preventing the spread of *A. invadans.*

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

Scoop net, cast net or seine net represent the best choices for catching diseased fish in natural waters or in fish ponds (FAO 2009).

Fish with characteristic EUS-like lesions should be sampled from affected populations

3.2. Selection of organs or tissues

The motile zoospore plays an important role in the spread of the disease. Once the motile spore attaches to the skin of the fish, the spore will germinate under suitable conditions and its hyphae will invade the fish skin, muscular tissue and reach the internal organs. Fish skeletal muscle is the target organ and exhibits major clinical signs of infection with *A. invadans* with mycotic granulomas (Iberahim *et al.,* 2018). Samples should not be taken from the middle of large lesions as these are likely to be devoid of visible and viable hyphae. Instead, samples should be taken from the leading edge of the infected area or lesion and where possible, multiple samples should be taken from an infected individual to obtain viable hyphae. Fungal hyphae can be seen in tissue squash mounts and histological sections at the leading edge of the infected area. Attempting to culture *A. invadans* from severe ulcers is often constrained because of contaminating bacteria, but still should be attempted. PCR on tissue taken from the leading edge of the ulcer also should be attempted.

3.3. Samples or tissues not suitable for pathogen detection

Samples should not be taken from the middle of large lesions as these are likely to be devoid of visible and viable hyphae.

3.4. Non-lethal sampling

None available.

3.5. Preservation of samples for submission

Fish specimens should be transported to the laboratory live or in ice-cooled boxes for further diagnosis. Samples must not be frozen since ~~the fungus~~ *A. invadans* is killed by freezing. Fish collected from remote areas should be anesthetised and can be fixed in normal 10% formalin or 10% phosphate-buffered formalin for at least 1–2 days. The fixed specimens are then transferred to double-layer plastic bags with formalin-moistened tissue paper.

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0 *General information* (*diseases of fish*).

3.5.1. Samples for pathogen isolation

The success of pathogen isolation depends strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples use alternative storage methods only after consultation with the receiving laboratory. Multiple samples should be taken from each lesion to increase the chances of obtaining viable hyphae.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1.

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard methods for histopathology can be found in Chapter 2.3.0.

3.5.4. Samples for other tests

None

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger animals should be processed and tested individually are available. However, smaller life stages (e.g. fry) can be pooled to provide a minimum amount of material for testing.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ = Methods are most suitable with desirable performance and operational characteristics.

++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
~~Squash mounts~~ Clinical signs	±	±	±	NA	+	+	+	NA				
Squash mounts					±	±	±	1	±	±	±	1
Histopathology					++	++	++	1	++	++	++	1
Cytopathology												
Cell or artificial media culture					++	++	++	1	+	+	+	1
Real-time PCR												
Conventional PCR					++	++	++	1				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation									++	++	++	1
Bioassay												
LAMP												
Ab ELISA												
Ag ELISA												
Other antigen detection methods												
Other method												

LV = level of validation, refers to the stage of validation in the OIE Pathway (Chapter 1.1.2); NA = not available; PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

Shading indicates the test is inappropriate or should not be used for this purpose.

~~Diagnosis of infection with *A. invadans* in clinically affected fish may be achieved by histopathology, oomycete isolation or polymerase chain reaction amplification.~~

4.1. Squash mounts

Aphanomyces invadans can be detected using microscopic examination of squash preparations prepared as follows:

i) Remove ulcer surface using a sharp scalpel blade.

ii) Cut the muscular tissue at the edge of the ulcer.

iii) Place the pieces of tissue on a cutting board then make thin slices using a sharp scalpel blade.

iv) Place the thinly sliced tissue between two glass slides and squeeze gently with fingers.

v) Remove one of the glass slides and cover the tissue with a cover-slip. View under a light microscope to find the nonseptate hyphae structure of *A. invadans* (12–25 μm in diameter).

4.2. Histopathology and cytopathology

Aphanomyces invadans can be detected using microscopic examination of fixed sections, prepared as follows:

i) Sample only live or moribund specimens of fish with clinical lesions.

ii) Take samples of skin/muscle (<1 cm^3), including the leading edge of the lesion and the surrounding tissue.

iii) Fix the tissues immediately in 10% formalin. The amount of formalin should be 10 times the volume of the tissue to be fixed.

4.2.1. Histological procedure

Standard methods for processing are provided in chapter 2.3.0. H&E and general fungus stains (e.g. Grocott's stain) will demonstrate typical granulomas and invasive hyphae.

4.2.2 Histopathological changes

Early lesions are caused by erythematous dermatitis with no obvious oomycete involvement. *Aphanomyces invadans* hyphae are observed growing in skeletal muscle as the lesions progress from a mild chronic active dermatitis to a severe locally extensive necrotising granulomatous dermatitis with severe floccular degeneration of the muscle. The oomycete elicits a strong inflammatory response and granulomas are formed around the penetrating hyphae.

4.3. Cell culture for isolation

4.3.1. Isolation of *Aphanomyces invadans* from internal tissues

The following are two methods of isolation of *A. invadans* adapted from Lilley *et al.* (1998) and Willoughby & Roberts (1994).

Method 1: Moderate, pale, raised, dermal lesions are most suitable for oomycete isolation attempts. Remove the scales around the periphery of the lesion and sear the underlying skin with a red-hot spatula so as to sterilise the surface. Using a sterile scalpel blade and sterile fine-pointed forceps, cut through the stratum compactum underlying the seared area and, by cutting horizontally and reflecting superficial tissues, expose the underlying muscle. Ensure the instruments do not make contact with the contaminated external surface and thereby contaminate the underlying muscle. Using aseptic techniques, carefully excise pieces of affected muscle, approximately 2 mm^3, and place on a Petri dish containing glucose/peptone (GP) agar (see Table 4.1) with penicillin G (100 units ml^{-1}) and streptomycin (100 μg ml^{-1}). Seal plates, incubate at room temperature or at 25°C and examine daily. Repeatedly transfer emerging hyphal tips on to fresh plates of GP agar with antibiotics until cultures are free of contamination.

Method 2: Lesions located on the flank or tail of fish <20 cm in length can be sampled by cutting the fish in two using a sterile scalpel and slicing a cross-section through the fish at the edge of the lesion. Flame the scalpel until red-hot and use this to sterilise the exposed surface of the muscle. Use a small-bladed sterile scalpel to cut

out a circular block of muscle (2–4 mm^3) from beneath the lesion and place it in a Petri dish of GP medium (see Table 4.1) with 100 units ml^{-1} penicillin G and 100 μg ml^{-1} streptomycin. Instruments should not contact the contaminated external surface of the fish. Incubate inoculated medium at approximately 25°C and examine under a microscope (preferably an inverted microscope) within 12 hours. Repeatedly transfer emerging hyphal tips to plates of GP medium with 12 g $litre^{-1}$ technical agar, 100 units ml^{-1} penicillin G and 100 μg ml^{-1} streptomycin until axenic cultures are obtained. The oomycete isolate can also be maintained at 25°C on glucose/yeast extract (GY) agar (see Table 4.1) and transferred to a fresh GY agar tube once every 1–2 weeks (Hatai & Egusa, 1979).

4.3.2. Identification of *Aphanomyces invadans*

Aphanomyces invadans does not produce any sexual structures and should thus not be diagnosed by morphological criteria alone. However, the oomycete can be identified to the genus level by inducing sporogenesis and demonstrating typical asexual characteristics of *Aphanomyces* spp., as described in Lilley *et al.,* 1998. *Aphanomyces invadans* is characteristically slow-growing in culture and fails to grow at 37°C on GPY agar (Table 4.1). Detailed temperature–growth profiles are given in Lilley & Roberts (1997). *A. invadans* can be identified by polymerase chain reaction (PCR) amplification of the rDNA of *A. invadans*.

4.3.3. Inducing sporulation in *Aphanomyces invadans* cultures

The induction of asexual reproductive structures is necessary for identifying oomycete cultures as members of the genus *Aphanomyces*. To induce sporulation, place an agar plug (3–4 mm in diameter) of actively growing mycelium in a Petri dish containing glucose/peptone/yeast (GPY) broth and incubate for 4 days at approximately 20°C. Wash the nutrient agar out of the resulting mat by sequential transfer through five Petri dishes containing autoclaved pond water (Table 4.1 2), and leave overnight at 20°C in autoclaved pond water. After about 12 hours, the formation of achlyoid clusters of primary cysts and the release of motile secondary zoospores should be apparent under the microscope.

Table 4.1 2. Media for isolation, growth and sporulation of* Aphanomyces invadans *cultures

GP (glucose/peptone) medium	GPY (glucose/peptone/ yeast) broth	GPY agar	GY agar (glucose/ yeast)	Autoclaved pond water
3 g $litre^{-1}$ glucose 1 g $litre^{-1}$ peptone 0.128 g $litre^{-1}$ $MgSO_4.7H_2O$ 0.014 g $litre^{-1}$ KH_2PO_4 0.029 g $litre^{-1}$ $CaCl_2.2H_2O$ 2.4 mg $litre^{-1}$ $FeCl_3.6H_2O$ 1.8 mg $litre^{-1}$ $MnCl_2.4H_2O$ 3.9 mg $litre^{-1}$ $CuSO_4.5H_2O$ 0.4 mg $litre^{-1}$ $ZnSO_4.7H_2O$	GP broth + 0.5 g $litre^{-1}$ yeast extract	GPY broth + 12 g $litre^{-1}$ technical agar	1% glucose, 0.25% yeast extract, 1.5% agar	Sample pond/lake water known to support oomycete growth. Filter through Whatman 541 filter paper. Combine one part pond water with two parts distilled water and autoclave. pH to 6–7.

Agent purification

Maintaining *A. invadans* in the axenic culture is necessary. As it is characteristically slow-growing, it easily becomes contaminated with other micro-organisms, such as bacteria and other fast-growing oomycetes and fungi. Attempts to purify or isolate *A. invadans* from contaminated cultures usually fail.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 2.5 *Use of molecular techniques for surveillance testing, confirmatory testing and diagnosis* of Chapter 2.3.0 *General information* (diseases of fish). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or running a gel.

4.4.1. Real-time PCR

No real-time PCR methods for detecting *A. invadans* in fish tissues are available.

4.4.2. Conventional PCR

DNA preparation from *A. invadans* isolate

DNA is extracted from an actively growing colony of *A. invadans* culture in GY broth at about 4 days or when young mycelia reach 0.5–1.0 cm in diameter. The mycelia are transferred to sterile 100-mm Petri dishes, washed twice with PBS and then placed on tissue paper for liquid removal. Hyphal tips (~50–250 mg) are excised with a sterile scalpel blade and transferred to a 1.5 ml microcentrifuge tube for DNA extraction. Commercial DNA extraction kits have been used successfully (Phadee *et al.*, 2004b; Vandersea *et al.*, 2006).

DNA preparation from *A. invadans*-infected tissue

Small pieces of *A. invadans*-infected tissue (25–50 mg) are suitable for DNA extractions (Phadee *et al.*, 2004a).

Diagnostic PCR technique

Three published techniques are specific to *A. invadans*.

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1 (Vandersea *et al.*, 2006) GenBank Accession No. AF396684, Product size 234bp)			
Aphanomyces invadans (ITS1)	Fwd Ainvad-2F: TCA-TTG-TGA-GTG-AAA-CGG-TG Rev Ainvad-ITSR1: GCT-AAG-GTT-TCA-GTA-TGT-AG	0.025 nM 0.025 nM	35 cycles: 95°C/30 sec, 56°C/45 sec,95°C/30 sec 72°C/2.5 min, 95°C/30 sec
Method 2 (Phadee *et al.*, 2004b) GenBank Accession No.AF396683, Product size 550bp)			
Aphanomyces invadans (ITS1-ITS2)	Fwd ITS11: GCC-GAA-GTT-TCG-CAA-GAA-AC Rev ITS23: CGT-ATA-GAC-ACA-AGC-ACA-CCA	500 nM 500 nM	35 cycles: 94°C/30 sec, 65°C/45 sec, 72°C/1 min
Method 3 (Oidtmann *et al.*, 2008) GenBank Accession No. EU422990 Product size 564bp)			
Aphanomyces invadans (ITS1-ITS2)	Fwd BO73: CTT-GTG-CTG-AGC-TCA-CAC-TC Rev BO639: ACA-CCA-GAT-TAC-ACT-ATC-TC	600 nM 600 nM	35 cycles: 96°C/1 min, 58°C/1 min, 72°C/1 min

~~The species-specific forward primer site is located near the 3' end of the SSU (small subunit) gene and a species-specific reverse primer site is located in the ITS1 region for Ainvad-2F (5'-TCA-TTG-TGA-GTG-AAA CGG-TG-3') and Ainvad ITSR1 (5' GGC TAA GGT TTC AGT ATG TAG-3'). The PCR mixture contained 25 pM of each primer, 2.5 mM each deoxynucleoside triphosphate, 0.5 U of Platinum *Taq* DNA polymerase and 20 ng of genomic DNA (either from an *Aphanomyces* isolate or from infected tissue) for a total volume of 50 µl. DNA is amplified in a thermocycle machine under the following cycle conditions: 2 minutes at 95°C; 35 cycles, each consisting of 30 seconds at 95°C, 45 seconds at 56°C, 2.5 minutes at 72°C; and a final extension of 5 minutes at 72°C. The PCR product is analysed by agarose gel electrophoresis and the target product is 234 bp (Vandersea *et al.*, 2006).~~

~~*Method 2*~~

~~The species-specific primer sites are located in the ITS1 and ITS2 regions. The forward primer is ITS11 (5'-GCC-GAA-GTT-TCG-CAA-GAA-AC-3') and the reverse is ITS23 (5'-CGT-ATA-GAC-ACA-AGC-ACA-CCA-3'). The PCR mixture contains 0.5 µM of each primer, 0.2 mM each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 0.6 U of *Taq* DNA polymerase and 20 ng of genomic DNA (from an *Aphanomyces* isolate) for a total volume of 25 µl. The DNA is amplified under the following cycle conditions: 5 minutes at 94°C; 25 cycles, each consisting of 30 seconds at 94°C, 30 seconds at 65°C, 1 minute at 72°C; and a final extension of 5 minutes at 72°C. The PCR product is analysed by agarose gel electrophoresis and the target product is~~

~~550 bp. PCR amplification using the DNA template from the infected tissue is similar to the above protocol except that 5 ng of the DNA template is used for 35 cycles (Phadee *et al.*, 2004b).~~

~~*Method 3*~~

~~The species-specific primer sites are located in the ITS1 and ITS2 regions. The forward primer is BO73 (5'-CTT-GTG-CTG-AGC-TCA-CAC-TC-3') and the reverse is BO639 (5'-ACA-CCA-GAT-TAC-ACT-ATC-TC-3'). The PCR mixture contains 0.6 µM of each primer, 0.2 mM of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 0.625 units of Taq DNA polymerase, and approximately 5 ng of genomic DNA (or 2.5 µl of DNA template extracted from 25 mg of infected tissue and suspended in 100 µl buffer) in a 50 µl reaction volume (Oidtmann *et al.*, 2008). The DNA is amplified under the following cycle conditions: 96°C for 5 minutes; 35 cycles of 1 minute at 96°C, 1 minute at 58°C and 1 minute at 72°C; followed by a final extension at 72°C for 5 minutes (Oidtmann, pers. comm.). The PCR product is analysed by agarose gel electrophoresis and the target product is 564 bp.~~

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~

4.4.3. Other nucleic acid amplification methods

None.

4.5. Amplicon sequencing

Nucleotide sequencing of all conventional PCR amplicons (Section 4.4.2) is recommended as one of the final steps for confirmatory diagnosis. *Aphanomyces invadans*-specific sequences will share a high degree of nucleotide similarity to one of the published reference sequences for *A. invadans* (Genbank accession AF396684).

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced, analysed and compared with published sequences.

4.6. *In-situ* hybridisation

A fluorescent peptide nucleic acid *in-situ* hybridisation (FISH) technique has demonstrated a high specificity for *A. invadans.* The technique can directly detect the mycelia-like structure of the oomycete in thinly sliced tissues of affected organs of susceptible fish. The fluorescein (FLU) probe designed to hybridise the small subunit of the rRNA *A. invadans* (bp 621 to 635; GenBank acc. AF396684) is 5'-FLU-GTA-CTG-ACA-TTT-CGT-3' or Ainv-FLU3.

The *A. invadans*-affected tissue is fixed and hybridised as soon as possible after the fish are collected to minimise RNA degradation. Tissue (~20 mg) is dissected from the periphery of the lesions with sterile scalpel blades and placed in individual wells of a 24-well microtitre plate. One ml ethanol-saline fixative (44 ml of 95% ethanol, 10 ml of deionised H_2O, and 6 ml of 25 × SET buffer [3.75 M NaCl, 25 mM EDTA (ethylene diamine tetra-acetic acid), 0.5 M Tris/HCl, pH 7.8]) containing 3% polyoxyethyl-enesorbitan monolaurate (Tween 20) is added to enhance tissue permeabilisation. The microtitre plate is gently agitated at room temperature on an orbital shaker (30 rpm) for 1.5 hours. The fixed tissues are rinsed (twice for 15 minutes each time) with 0.5 ml of hybridisation buffer (5 × SET, 0.1% [v/v] Igepal-CA630 and 25 µg ml^{-1} poly[A]) containing 3% Tween 20. The hybridisation buffer is removed, and the tissues are resuspended in 0.5 ml of hybridisation buffer containing 3% Tween 20 and 100 nM Ainv-FLU3 probe. "No-probe" control specimens are incubated with 0.5 ml of hybridisation buffer/3% Tween 20. All tissues are incubated at 60°C for 1 hour in the dark. Following incubation, the tissues are rinsed twice with 1 ml of pre-warmed (60°C) 5 × SET buffer containing 3% Tween 20 to remove residual probe. The tissue specimens are mounted onto poly-L-lysine-coated microscope slides. One drop of the light anti-fade solution is placed on the specimens, which are then overlaid with a cover-slip. Analyses are performed by light and epifluorescence microscopy. The camera and microscope settings for epifluorescent analyses are held constant so that comparative analyses of relative fluorescence intensity can be made between probed and non-probed specimens. The fluorescent oomycete hyphae appear as green fluorescence against the dark tissue background. The above detailed protocols are published by Vandersea *et al.* (2006). Using the FISH technique, *A. invadans* can be visualised very well in thinly sliced tissue compared with freshly squashed tissue.

4.7. Immunohistochemistry

None.

4.8. Bioassay

Fish can be experimentally infected by intramuscular injection of 0.1 ml suspension of 100+ motile zoospores into fish susceptible to infection with *A. invadans* at 20°C. Histological growth of aseptate hyphae, 12–25 μm in diameter, should be demonstrated in the muscle of fish sampled after 7 days, and typical mycotic granulomas should be demonstrated in the muscle of fish sampled after 10–14 days.

4.9. Antibody or antigen detection methods

Polyclonal antibodies against *A. invadans* or *Aphanomyces* saprophyte showed cross-reactivity to each other using protein gel electrophoresis and Western blot analysis and immunohistochemistry. (Lilley *et al.*, 1997b). However, a specific monoclonal antibody against *A. invadans* ~~developed later~~ was found to have high specificity and high sensitivity to *A. invadans* using immunofluorescence. This monoclonal antibody could detect *A. invadans* hyphae at the early stage of infection (Miles *et al.*, 2003).

A monoclonal antibody-based flow-through immunoassay was developed by Adil *et al*. (2013). This assay was found to have high analytical (0.007mg ml^{-1}) and diagnostic specificity comparable to PCR.

4.10. Other methods

Serological methods for detection and identification of *A. invadans* in diseased specimens are not practical. If necessary, the monoclonal antibody offers a better specificity and sensitivity than polyclonal antibody for serological detection or identification of *A. invadans* in diseased specimens or in pathogen isolates.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The test for targeted surveillance to declare freedom from infection with *A. invadans* is examination of target populations for gross signs of infection with *A. invadans*. Surveys should be conducted during seasons that favour clinical manifestation of infection with *A. invadans* or when water temperatures are in the range 18–25°C.

Using the gross sign test for targeted surveillance, a large sample of the fish population should be examined live with a sample size sufficient to meet survey design assumptions as described in Chapter 1.4 of the Aquatic Code.

If fish show gross signs consistent with infection with *A. invadans*, they should be categorised as suspect fish, and the location/farm/compartment/zone should be considered suspect. Suspect specimens should be further tested using the methods listed under presumptive diagnosis followed by confirmative diagnosis as described in the Table 4.1.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (6.1) or presence of clinical signs (6.2) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for suspect and confirmed cases have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent.

6.1. Apparently healthy animals or animals of unknown health status [13]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. ~~Geographical~~ Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy populations

[13] For example transboundary commodities.

The presence of infection with *A. invadans* shall be suspected if at least one of the following criteria is met:

i) Observation of clinical signs consistent with infection with *A. invadans* [14]

ii) A positive result obtained by any of the diagnostic techniques described in Section 4.

6.1.2. Definition of confirmed case in apparently healthy populations

The presence of infection with *A. invadans* is considered to be confirmed if one or more of the following criteria is met:

i) Histopathology consistent with infection with *A. invadans* and positive result by PCR and amplicon sequencing

ii) Histopathological changes consistent with infection with *A. invadans* and positive result for *in-situ* hybridisation

iii) Artificial media culture and positive result by PCR and sequencing of the amplicon

6.2 Clinically affected animals

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *A. invadans* shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with infection with *A. invadans* as described in this chapter, with or without elevated mortality

ii) Positive result by a recommended molecular detection test

iii) Histological changes consistent with infection with *A. invadans*

iv) Visual observation of hyphae characteristic (direct or by microscopy) of *A. invadans*

v) Culture and isolation of *A. invadans*-type colonies

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *A. invadans* is considered to be confirmed if one or more of the following criteria is met:

i) Visualisation of hyphae under squash mounts and a positive result by PCR and sequencing of the amplicon

ii) Histopathological changes consistent with infection with *A. invadans* and a positive result by PCR and sequencing of the amplicon

iii) Histopathological changes consistent with infection with *A. invadans* and positive result for *in-situ* hybridisation

iv) Artificial media culture and a positive result by PCR and sequencing of the amplicon

v) Positive result for *in-situ* hybridisation and a positive result by PCR and sequencing of the amplicon

6.3. Diagnostic sensitivity and specificity for diagnostic tests [under study]

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *A. invadans* is provided in Table 6.3.1. (**note**: no data are currently available). This information can be used for the design of surveys for infection with *A. invadans*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data is only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2 and the information is available within published diagnostic accuracy studies.

[14] Note that surveillance of apparently healthy populations for EUS is based on examination of target populations for clinical signs of infection with *A. invadans* (see Section 5).

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity.

7. References

ADIL B., SHANKAR K.M., NAVEEN KUMAR B.T., PATIL R., BALLYAYA A., RAMESH K.S., POOJARY S.R., BYADGI O.V. & SIRIYAPPAGOUDER P. (2013). Development and standardization of a monoclonal antibody-based rapid flow-through immunoassay for the detection of *Aphanomyces invadans* in the field. *J. Vet. Sci.*, **14**, 413–419.

AFZALI S.F., HASSAN M.D., ABDUL-RAHIM A.M., SHARIFPOUR I. & SABRI J. (2013). Isolation and identification of *Aphanomyces* species from natural water bodies and fish farms in Selangor, Malaysia. *Malaysian Appl. Biol.*, **42**, 21–31.

ANDREW T., HUCHZERMEYER K., MBEHA B. & NENGU S. (2008). Epizootic ulcerative syndrome affecting fish in the Zambezi river system in Southern Africa. *Vet. Rec.,* **163**, 629–632.

BALDOCK F.C., BLAZER V., CALLINAN R., HATAI K., KARUNASAGAR I., MOHAN C.V. & BONDAD-REANTASO M.G. (2005). Outcomes of a short expert consultation on epizootic ulcerative syndrome (EUS): Re-examination of causal factors, case definition and nomenclature. *In:* Diseases in Asian Aquaculture V, Walker P., Lester R. & Bondad-Reantaso M.G., eds. Fish Health Section, Asian Fisheries Society, Manila, Philippines, 555–585.

BALASURIYA L.K.S.W. (1994). Epizootic ulcerative syndrome in fish in Sri Lanka, country status report. *In:* Proceeding of the ODA Regional Seminar on Epizootic Ulcerative, Robert R.J., Campbell B. & MacRae I.H., eds. Aquatic Animal Health Research Institute, Bangkok, Thailand, pp 39–47.

BLAZER V.S., VOGELBEIN W.K., DENSMORE C.L., MAY E.B., LILLEY J.H. & ZWERNER D.E. (1999). Aphanomyces as a cause of ulcerative skin lesions of menhaden from Chesapeake Bay tributaries. *J. Aquat. Anim. Health,* **11**, 340–349.

BONDAD-REANTASO M.G., LUMANLAN S.C., NATIVIDAD J.M. & PHILLIPS M.J. (1992). Environmental monitoring of the epizootic ulcerative syndrome (EUS) in fish from Munoz, Nueva Ecija in the Philippines. *In:* Diseases in Asian Aquaculture 1, Shariff M., Subasinghe R.P. & Arthur J.R., eds. Fish Health Section, Asian Fisheries Society, Manila, The Philippines, 475–490.

CATAP E.S. & MUNDAY B.L. (1998). Effects of variations of water temperature and dietary lipids on the expression of experimental epizootic ulcerative syndrome (EUS) in sand whiting, Sillago ciliata. *Fish Pathol.,* **33**, 327–335.

CAVALIER-SMITH T. & CHAO E.E.Y. (2006). Phylogeny and Megasystematics of Phagotrophic Heterokonts (Kingdom Chromista). *J. Mol. Evol.,* **62**, 388–420.

CHINABUT S. & ROBERTS R.J. (1999). Pathology and Histopathology of Epizootic Ulcerative Syndrome (EUS). Aquatic Animal Health Research Institute, Department of Fisheries, Royal Thai Government, Bangkok, Thailand, 33 pp. ISBN 974-7604-55-8.

CHINABUT S., ROBERTS R.J., WILLOUGHBY G.R. & PEARSON M.D. (1995) Histopathology of snakehead, *Channa striatus* (Bloch), experimentally infected with the specific *Aphanomyces* fungus associated with epizootic ulcerative syndrome (EUS) at different temperatures. *J. Fish Dis.*, **18**, 41–47.

CRUZ-LACIERDA E.R. & SHARIFF M. (1995). Experimental transmission of epizootic ulcerative syndrome (EUS) in snakehead, *Ophicephalus striatus. Dis. Asian Aquac.*, **II**., 327–336.DIEGUEZ-URIBEONDO J., GARCIA M.A., CERENIUS L., KOZUBÍKOVÁ E., BALLESTEROS I., WINDELS C., WEILAND J., KATOR H., SÖDERHÄLL K. & MARTÍN M.P. (2009). Phylogenetic relationships among plant and animal parasites, and saprotrophs in *Aphanomyces* (Oomycetes). *Fungal Genetics and Biology*, **46**, 365–376.

EGUSA S. & MASUDA N. (1971). A new fungal disease of Plecoglossus altivelis. *Fish Pathol.,* **6**, 41–46.

EUROPEAN FOOD SAFETY AUTHORITY EFSA (2011a). Scientific Opinion on Epizootic Ulcerative Syndrome. *EFSA J.*, **9**, 2387.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2011b). Report of the technical hearing meeting on Epizootic Ulcerative Syndrome (EUS). *EFSA Support. Publ.*, **8**, 1–16.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO) (1986). Report of the expert consutation on ulcerative fish diseases in the Asia-Pacific region (TCP/RAS/4508). Bangkok, August 1986. FAO, Regional Office for Asia and the Pacific, Bangkok, Thailand.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO) (2009). Report of the international emergency disease investigation task force on a serious fish disease in Southern Africa, 18–26 May 2007, FAO, Rome, Italy, 70 pp.

FRASER G.C., CALLINAN R.B. & CALDER L.M. (1992). *Aphanomyces* species associated with red spot disease: an ulcerative disease of estuarine fish from eastern Australia. *J. Fish Dis.*, **15**, 173–181.

GOMO C., HANYIRE T., MAKAYA P. & SIBANDA S. (2016). Outbreak of epizootic ulcerative syndrome (EUS) in Seranochromis robustus fish spesies in Darwendale dam, Zimbabwe. *African J. Fish. Sci.*, **4**, 204–205.

HANJAVANIT C. (1997). Mycotic granulomatosis found in two species of ornamental fishes imported from Singapore. *Mycoscience*, 38, 433–436.

HATAI K. & EGUSA S. (1979). Studies on pathogenic fungus of mycotic granulomatosis III. Development of the medium for MG-fungus. *Fish Pathol.,* **13**, 147–152.

HATAI K., EGUSA S., TAKAHASHI S. & OOE K. (1977). Study on the pathogenic fungus of mycotic granulomatosis – I. Isolation and pathogenicity of the fungus from cultured-ayu infected with the disease. *Fish Pathol.,* **12**, 129–133.

HATAI K., NAKAMURA K., AN RHA S., YUASA K. & WADA S. (1994). *Aphanomyces* infection in dwarf gourami (*Colisa lalia*). *Fish Pathol.,* **29**, 95–99.

HAWKE J.P., GROOTERS A.M. & CAMUS A.C. (2003). Ulcerative Mycosis Caused by *Aphanomyces invadans* in Channel Catfish, Black Bullhead, and Bluegill from Southeastern Louisiana. *J. Aquat. Anim. Health.*, **15**, 120–127.

HERBERT B., JONES J.B.B., MOHAN C.V. V. & PERERA R.P.P. (2019). Impacts of epizootic ulcerative syndrome on subsistence fisheries and wildlife. *Rev. Sci. Tech.,* **38**, 459–475.

HUCHZERMEYER C.F., HUCHZERMEYER K.D.A., CHRISTISON K.W., MACEY B.M., COLLY P.A., HANG'OMBE B.M. & SONGE M.M. (2018). First record of epizootic ulcerative syndrome from the Upper Congo catchment: An outbreak in the Bangweulu swamps, Zambia. *J. Fish Dis.*, **41**, 87–94.

HUCHZERMEYER K.D.A. & VAN DER WAAL B.C.W. (2012). Epizootic ulcerative syndrome: Exotic fish disease threatens Africa's aquatic ecosystems. *J. S. Afr. Vet. Assoc.*, **83**, 1–6.

IBERAHIM N.A., TRUSCH F. & VAN WEST P. (2018). *Aphanomyces invadans*, the causal agent of Epizootic Ulcerative Syndrome, is a global threat to wild and farmed fish. *Fungal Biol. Rev.,* **44**, 1–13.

KHAN M.H., MARSHALL L., THOMPSON K.D., CAMPBELL R.E. & LILLEY J.H. (1998). Susceptibility of five fish species (Nile tilapia, rosy barb, rainbow trout, stickleback and roach) to intramuscular injection with the *Oomycete* fish pathogen, *Aphanomyces invadans*. *Bull. Eur. Assoc. Fish Pathol.,* **18**, 192–197.

KIRYU Y., SHIELDS J.D., VOGELBEIN W.K., KATOR H. & BLAZER V.S. (2003). Infectivity and pathogenicity of the oomycete *Aphanomyces invadans* in Atlantic menhaden *Brevoortia tyrannus. Dis. Aquat. Org.*, **54**, 135–146.

KUMAR P., SARKAR P., STEFI RAJU V., MANIKANDAN V., GURU A., ARSHAD A., ELUMALAI P. & AROCKIARAJ J. (2020). Pathogenicity and Pathobiology of Epizootic Ulcerative Syndrome (EUS) Causing Fungus *Aphanomyces invadans* and Its Immunological Response in Fish. *Rev. Fish. Sci. Aquac.*, **28**, 358–375.

LILLEY J.H., CALLINAN R.B., CHINABUT S., KANCHANAKHAN S., MACRAE I.H. & PHILLIPS M.J., ~~FALLIS A., LILLEY J.H., CALLINAN R.B., CHINABUT S., KANCHANAKHAN S., MACRAE I.H. & PHILLIPS M.J.~~ (1998). Epizootic ulcerative syndrome (EUS) technical handbook. Bangkok: The Aquatic Animal Health Research Institute.

LILLEY J.H., HART D., PANYAWACHIRA V., KANCHANAKHAN S., CHINABUT S., SÖDERHÄLL K. & CERENIUS L. (2003). Molecular characterization of the fish-pathogenic fungus Aphanomyces invadans. *J. Fish Dis.,* **26**, 263–275.

LILLEY J.H., HART D., RICHARDS R.H., ROBERTS R.J., CERENIUS L. & SODERHALL K. (1997a). Pan-Asian spread of single fungal clone results in large scale fish kills. *Vet. Rec.*, **140**, 653–654.

LILLEY J.H., PETCHINDA T. & PANYAWACHIRA V. (2001). *Aphanomyces invadans* zoospore physiology: 4. *In vitro* viability of cysts. *The AAHRI Newsletter*, **10**, 1–4.

LILLEY J.H. & ROBERTS R.J. (1997). Pathogenicity and culture studies comparing the *Aphanomyces* involved in epizootic ulcerative syndrome (EUS) with other similar fungi. *J. Fish Dis.*, **20**, 135–144.

LILLEY J.H., THOMPSON K.D. & ADAMS A. (1997b). Characterization of *Aphanomyces invadans* by electrophoretic and Western blot analysis. *Dis. Aquat. Org.*, **30**, 187–197.

LUMANLAN-MAYO S.C., CALLINAN R.B., PACLIBARE J.O., CATAP E.S. & FRASER, G.C. (1997). Epizootic ulcerative syndrome (EUS) in rice-fish culture systems: an overview of field experiments 1993-1995. *In:* Diseases in Asian Aquaculture III, Flegel T.W. & MacRae I.H., eds. Fish Health Section, Asian Fisheries Society, Manila, The Philippines, 129–138.

MCHUGH K.J., CHRISTISON K.W., WEYL O.L.F. & SMIT N.J. (2014). Histological Confirmation of Epizootic Ulcerative Syndrome in Two Cyprinid Species from Lake Liambezi, Zambezi Region, Namibia. *African Zool.*, **49**, 311–316.

MCKENZIE R.A. & HALL W.T.K. (1976). Dermal ulceration of mullet (*Mugil cephalus*). *Aust. Vet. J.,* **52**, 230–231.

MILES D.J.V., POLCHANA J., LILLEY J.H., KANCHANAKHAN S., THOMPSON K.D. & ADAMS A. (2001). Immunostimulation of striped snakehead *Channa striata* against epizootic ulcerative syndrome. *Aquaculture*, **195**, 1–15.

MILES D.J.C., THOMPSON K.D., LILLEY J.H. & ADAMS A. (2003). Immunofluorescence of the epizootic ulcerative syndrome pathogen, *Aphanomyces invadans*, using a monoclonal antibody. *Dis. Aquat. Org.*, **55**, 77–84.

NOGA E.J. & DYKSTRA M.J. (1986). Oomycete fungi assocaited with ulcerative mycosis in menhaden, *Brevoortia tyrannus* (Latrobe). *J. Fish Dis.*, **9**, 47–53.

OIDTMANN B. (2012). Review of biological factors relevant to import risk assessments for epizootic ulcerative syndrome (*Aphanomyces invadans*). *Transbound. Emerg. Dis.*, **59**, 26–39.

OIDTMANN B., STEINBAUER GEIGER S. & HOFFMANN R.W. (2008). Experimental infection and detection of *Aphanomyces invadans* in European catfish, rainbow trout and European eel. *Dis. Aquat. Org.*, **82**, 185–207.

PAGRUT N.K., GANGULY S., JAISWAL V. & SINGH C. (2017). An overview on epizootic ulcerative syndrome of fishes in India: A comprehensive report. *J. Entomol. Zool. Stud.*, **5**, 1941–1943.

PHADEE P., KURATA O. & HATAI K. (2004a). A PCR method for the detection of *Aphanomyces piscicida*. *Fish Pathol.,* **39**, 25–31.

PHADEE, P., KURATA, O., HATAI K., HIRONO I. & AOKI T. (2004b). Detection and identification of fish-pathogenic *Aphanomyces piscicida* using polymerase chain reaction (PCR) with species-specific primers. *J. Aquat. Anim. Health*, **16**, 220–230.

PRADHAN P.K., MOHAN C.V., SHANKAR K.M., KUMAR B.M. & DEVARAJA G. (2007). Yearlings of Indian major carps resist infection against the epizootic ulcerative syndrome pathogen, *Aphanomyces invadans*. *Current Science*, **92**, 1430–1434.

TONGUTHAI K. (1985). A preliminary account of ulcerative fish diseases in the Indo-Pacific region (a comprehensive study based on Thai experiences). National Inland Fisheries Institute, Bangkok, Thailand, 39 pp.

TSUI C.K.M., MARSHALL W., YOKOYAMA R., HONDA D., LIPPMEIER J.C., CRAVEN K.D., PETERSON P.D. & BERBEE M.L. (2009). Labyrinthulomycetes phylogeny and its implications for the evolutionary loss of chloroplasts and gain of ectoplasmic gliding. *Mol. Phylogenet. Evol.*, **50**, 129–140.

VANDERSEA M.W., LITAKER R.W., YONNISH B., SOSA E., LANDSBERG J.H., PULLINGER C., MOON-BUTZIN P., GREEN J., MORRIS J.A., KATOR H., NOGA E.J. & TESTER P.A. (2006). Molecular assays for detecting *Aphanomyces invadans* in ulcerative mycotic fish lesions. *Appl. Environ. Microbiol.,* **72**, 1551–1557.

VISHWANATH T., MOHAN C. & SHANKAR K. (1998). Epizootic Ulcerative Syndrome (EUS), associated with a fungal pathogen, in Indian fishes: histopathology – 'a cause for invasiveness'. *Aquaculture*, **165**, 1–9.

WADA S., AN RHA S., KONDOH T., SUDA H., HATAI K. & ISHII H. (1996). Histopathological comparison between ayu and carp artificially infected with *Aphanomyces piscicida. Fish Pathol.,* **31**, 71–80.

WILLOUGHBY L.G. & ROBERTS R.J. (1994). Improved methodology for isolation of the *Aphanomyces* fungal pathogen of epizootic ulcerative syndrome (EUS) in Asian fish. *J. Fish Dis.,* **17**, 541–543.

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* *

NB: There is currently (2022) no OIE Reference Laboratories for infection with *Aphanomyces invadans* (please consult the OIE web site for the most up-to-date list: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

NB: FIRST ADOPTED IN 1995 AS EPIZOOTIC ULCERATIVE SYNDROME; MOST RECENT UPDATES ADOPTED IN 2013.

CHAPTER 2.3.2.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

Infection with epizootic haematopoietic necrosis virus means infection with the pathogenic agent *epizootic haematopoietic necrosis virus* (EHNV) of the Genus *Ranavirus* of the Family *Iridoviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

EHNV is a species of the genus *Ranavirus* in the Family *Iridoviridae* (Chinchar *et al.,* 2005). In addition to fish, ranaviruses have been isolated from healthy or diseased frogs, salamanders and reptiles in America, Europe and Australia (Chinchar*,* 2002; Drury *et al.,* 2002; Fijan *et al.,* 1991; Hyatt *et al*., 2002; Speare & Smith, 1992; Whittington *et al.*, 2010; Wolf *et al*., 1968; Zupanovic *et al*., 1998). Ranaviruses have large (150–180 nm), icosahedral virions, a double-stranded DNA genome 150–170 kb, and replicate in both the nucleus and cytoplasm with cytoplasmic assembly (Chinchar *et al.,* 2005).

Since the recognition of disease due to EHNV in Australia in 1986, similar systemic necrotising iridovirus syndromes have been reported in farmed fish. These include catfish (*Ictalurus melas*) in France (European catfish virus, ECV) (Pozet *et al*., 1992), sheatfish (*Silurus glanis*) in Germany (European sheatfish virus, ESV) (Ahne *et al.,* 1989; 1990), turbot (*Scophthalmus maximus*) in Denmark (Bloch & Larsen, 1993), and cod (*Gadus morhua*) in Denmark (Cod iridovirus, CodV) (Ariel *et al.,* 2010). EHNV, ECV, ESV, and CodV share >98% nucleotide identity across concatenated sequences across the RNR-α, DNApol, RNR-β, RNAse II and MCP gene regions (Ariel *et al*., 2010).

EHNV and ECV can be differentiated using genomic analysis (Ahne *et al.,* 1998; Holopainen *et al*., 2009; Hyatt *et al*., 2000; Mao *et al*., 1996; 1997; Marsh *et al*., 2002). This enables epidemiological separation of disease events in finfish in Australia (EHNV) and Europe (ECV), and differentiation of these from ranavirus occurrences in amphibians.

2.1.2. Survival and stability in processed or stored samples

EHNV can persist in frozen fish tissues for more than 2 years (Langdon, 1989) and frozen fish carcases for at least a year (Whittington *et al.*, 1996).

2.1.3. Survival and stability outside the host

EHNV is resistant to drying and remained infective for 97 days at 15°C and 300 days at 4°C in water (Langdon, 1989). For these reasons, it is presumed that EHNV would persist for months to years on a fish farm in water and sediment, as well as on plants and equipment.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with EHNV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Esocidae	*Esox lucius*	Northern pike
Galaxiidae	*Galaxias olidus*	Mountain galaxias
Ictaluridae	*Ameiurus melas*	Black bullhead
Melanotaeniidae	*Melanotaenia fluviatilis*	Crimson spotted rainbow fish
Percidae	*Perca fluviatilis*	European perch
	Sander lucioperca	Pike-perch
Percichthyidae	*Macquaria australasica*	Macquarie perch
Poeciliidae	*Gambusia holbrooki*	Eastern mosquito fish
	Gambusia affinis	Mosquito fish
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout
Terapontidae	*Bidyanus bidyanus*	Silver perch

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with EHNV according to Chapter 1.5 of the *Aquatic Code* are: none known.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: Atlantic salmon (*Salmo salar*), freshwater catfish (*Tandanus tandanus*), golden perch (*Macquaria ambigua*), Murray cod (*Maccullochella peelii*) and purple spotted gudgeon (*Mogurnda adspersa*).

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Natural infections and disease have been limited to European perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) in Australia. The disease is more severe in European perch and in juveniles compared with adult fish (Whittington *et al.,* 2010). There are no descriptions of infection of eggs or early life stages of any other fish species.

For the purposes of Table 4.1, larvae and fry up to approximately 5 g in weight may be considered to be early life stages, fingerlings and grower fish up to 500 g may be considered to be juveniles, and fish above 500 g may be considered to be adults.

2.2.4. Distribution of the pathogen in the host

Target organs and tissues infected with the virus are kidney, spleen and liver. It is not known if EHNV can be detected in gonadal tissues, ovarian fluid or milt or whether these tissues are suitable for surveillance of broodstock.

2.2.5. Aquatic animal reservoirs of infection

Rainbow trout: The high case fatality rate and low prevalence of infection with EHNV in natural infections in rainbow trout means that the recruitment rate of carriers is likely to be very low (<2%) (Whittington *et al.,* 1994). EHNV has been detected in growout fish but histopathological lesions consistent with infection with EHNV indicated an active infection rather than a carrier state (Whittington *et al.,* 1999). Anti-EHNV serum antibodies were not detected in fingerlings during or after an outbreak but were detected in a low proportion of growout fish, hence, it is uncertain whether these were survivors of the outbreak (Whittington *et al.,* 1994; 1999). There are data for European stocks of rainbow trout in experimental infections where potential carriers were identified (Ariel & Bang Jensen, 2009).

European perch: EHNV was isolated from 2 of 40 apparently healthy adult European perch during epizootics in juveniles in Victoria, Australia (Langdon & Humphrey, 1987), but as the incubation period extends for up to 28 days (Whittington & Reddacliff, 1995), these fish may have been in the preclinical phase.

2.2.6. Vectors

None demonstrated. ~~Birds are potential vectors for EHNV, it being carried in the gut, on feathers, feet and the bill (Whittington *et al.*, 1996).~~

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Rainbow trout: It appears that under natural farm conditions EHNV is poorly infective but once infected, most fish succumb to the disease ~~has a high case fatality rate~~. Infection with EHNV may be present on a farm without causing suspicion because the mortality rate may not rise above the usual background rate. Infection with EHNV has most often been reported in young fingerlings <125 mm fork length with daily mortality of less than 0.2% and total mortality of up to 4%. However, rainbow trout of all ages may be susceptible, although infection has not yet been seen in broodstock (Whittington *et al.*, 1994; 1999). There is a low direct economic impact because of the low mortality rate. Differences in susceptibility between European and Australian stocks of rainbow trout may exist (Ariel & Bang Jensen, 2009).

European perch: There is a very high rate of infection and mortality in natural outbreaks that, over time, leads to loss in wild fish populations (Langdon & Humphrey, 1987; Langdon *et al.,* 1986; Whittington *et al.*, 1996). Experimental bath inoculation with as few as 0.08 $TCID_{50}$ ml^{-1} was lethal, and doses too low to be detected by virus isolation in BF-2 cells were fatal by intraperitoneal inoculation (Whittington & Reddacliff, 1995). Differences in susceptibility between European and Australian stocks of European perch may exist (Ariel & Bang Jensen, 2009).

2.3.2. Clinical signs, including behavioural changes

Moribund fish may have loss of equilibrium, flared opercula and may be dark in colour (Reddacliff & Whittington, 1996). Clinical signs are usually more obvious in fingerlings and juvenile fish than adults of both rainbow trout and European perch. There may be clinical evidence of poor husbandry practices, such as overcrowding and suboptimal water quality, manifesting as skin, fin and gill lesions (Reddacliff & Whittington, 1996).

2.3.3 Gross pathology

There may be no gross lesions in affected fish. A small proportion of fish may have enlargement of kidney, liver or spleen. There may be focal white to yellow lesions in the liver corresponding to areas of necrosis (Reddacliff & Whittington, 1996).

2.3.4. Modes of transmission and life cycle

Rainbow trout: EHNV has spread between rainbow trout farms by transfer of infected fingerlings and probably transport water (Langdon *et al.,* 1988; Whittington *et al.*, 1994; 1999). The low prevalence of infection in rainbow trout means that active infection can easily go unrecognised in a population and be spread by trading fish. There are no data on possible vertical transmission of EHNV on or within ova, and disinfection protocols for ova have not been evaluated. EHNV has not yet been isolated from ovarian tissues or from broodstock. Annual recurrence in farmed rainbow trout may be due to reinfection of successive batches of fish or from wild European perch present in the same catchment.

European perch: The occurrence of infection with EHNV in European perch in widely separated river systems and impoundments suggested that EHNV was spread by translocation of live fish or bait by recreational fishers (Whittington *et al.,* 2010).

The route of infection is unknown. European perch and rainbow trout are susceptible to immersion exposure. The virus infects a range of cell types including hepatocytes, haematopoietic cells and endothelial cells in many organs (Reddacliff & Whittington, 1996). Virus is shed into water from infected tissues and carcasses as they disintegrate.

2.3.5. Environmental factors

Rainbow trout: Outbreaks appear to be related to poor husbandry, particularly overcrowding, inadequate water flow and fouling of tanks with feed. Damage to skin may provide a route of entry for EHNV. Outbreaks have been seen on farms at water temperatures ranging from 11 to 20°C (Whittington *et al.*, 1994; 1999). The incubation period after intraperitoneal inoculation was 3–10 days at 19–21°C compared with 14–32 days at 8–10°C (Whittington & Reddacliff, 1995).

European perch: Natural epizootics of infection with EHNV affecting juvenile and adult European perch occur mostly in summer (Langdon & Humphrey, 1987; Langdon *et al.,* 1986; Whittington *et al.*, 1994). It has been assumed that the disease in juvenile fish is related to the annual appearance of large numbers of non-immune young fish and their subsequent exposure to the virus while schooling in shallow waters; adults are uncommonly involved in these outbreaks. It is possible that environmental temperature is the trigger for outbreaks as juvenile fish feed in warm shallow waters on planktonic fauna, whereas adults feed on benthic invertebrates and larger prey in deeper cooler water (Whittington & Reddacliff, 1995). Experimentally, the incubation period ranged from 10 to 28 days at 12–18°C compared with 10–11 days at 19–21°C, and adult perch were refractory to infection at temperatures below 12°C (Whittington & Reddacliff, 1995). European stocks of European perch also displayed temperature-dependent susceptibility (Ariel & Bang Jensen, 2009).

2.3.6. Geographical distribution

Infection with EHNV has been reported from rainbow trout farms within two river catchments in New South Wales, Australia (Whittington *et al*., 2010). Infection with EHNV is endemic in south-eastern Australia, with a discontinuous distribution (Whittington *et al*., 2010).

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

Not available.

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy including blocking agents

None available.

2.4.3. Immunostimulation

None available.

2.4.4. Breeding resistant strains

There has been no formal breeding programme for resistant strains of susceptible species. However, experimental trials using a bath exposure have shown that European perch from water bodies in New South Wales, Australia with previous EHNV infections showed lower mortality compared with European perch from neighbouring and distant water bodies in Australia that have no previous history of EHNV (Becker *et al.,* 2016).

2.4.5. Inactivation methods

EHNV is susceptible to 70% ethanol, 200 mg litre^{-1} sodium hypochlorite or heating to 60°C for 15 minutes (Langdon, 1989). Data for the inactivation of amphibian ranavirus may also be relevant: 150 mg/litre chlorhexidine and 200 mg/litre potassium peroxymonosulphate were effective after 1 minute contact time (Bryan *et al*., 2009). If it is first dried, EHNV in cell culture supernatant is resistant to heating to 60°C for 15 minutes (Whittington *et al*., 2010).

2.4.6. Disinfection of eggs and larvae

Not tested.

2.4.7. General husbandry

Disease control in rainbow trout at the farm level relies on reducing the impact of infection by maintaining low stocking rates and adequate water quality. Investigations on one rainbow trout farm indicated that ponds with high stocking rates and low water flow, and thus poorer water quality, may result in higher levels of clinical disease compared with ponds on the same farm with lower stocking rates and higher water flow (Whittington *et al.,* 1994). The mechanism of protection may be through maintenance of healthy integument (Whittington *et al.,* 1994).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples which are most likely to be infected.

3.1. Selection of populations and individual specimens

Clinical inspections should be carried out during a period when water temperature is conducive to development of clinical disease (see Section 2.3.5). All production units (ponds, tanks, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. For the purposes of disease surveillance, fish to be sampled are selected as follows:

i) The most susceptible species (e.g. rainbow trout and European perch) should be sampled preferentially. Other susceptible species listed in Section 2.2.1 should be sampled proportionally.

ii) Risk-based criteria should be employed to preferentially sample lots or populations with a history of abnormal mortality, potential exposure events or where there is evidence of poor water quality or husbandry. If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) If weak, abnormally behaving or freshly dead fish are present, such fish should be selected. If such fish are not present, the fish selected should include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

For disease outbreak investigations, moribund fish or fish exhibiting clinical signs of infection with EHNV should be collected. Ideally fish should be collected while alive, however recently dead fish can also be selected for diagnostic testing. It should be noted however, that there will be a significant risk of contamination with environmental bacteria if the animals have been dead for some time.

3.2. Selection of organs or tissues

Liver, anterior kidney and spleen from individual fish are pooled (Jaramillo *et al.,* 2012).

3.3. Samples or tissues not suitable for pathogen detection

Inappropriate tissues include gonads, gonadal fluids, milt and ova, ~~since~~ because there is no evidence of reproductive tract infection.

3.4. Non-lethal sampling

~~No~~ Non-lethal samples (blood, fin, gill, integument or mucous) are unsuitable for testing ~~EHNV~~.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0.

3.5.1. Samples for pathogen isolation

For recommendations on transporting samples for virus isolation to the laboratory, see Section B.2.4 of Chapter 2.3.0 *General information* (*diseases of fish*).

3.5.2. Preservation of samples for molecular detection

~~Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.~~ Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.2.5 of Chapter 2.3.0. *General information* (*diseases of fish*).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

~~Tissue samples for histopathology should be fixed immediately after collection in 10% neutral buffered formalin. The recommended ratio of fixative to tissue is 10:1.~~ Standard sample collection, preservation and processing methods for histological techniques can be found in Section 2.2 of Chapter 2.3.0 *General information* (diseases of fish).

3.5.4. Samples for other tests

Not recommended for routine diagnostic testing.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger fish should be processed and tested individually. Small life stages such as fry or specimens can be pooled to provide the minimum amount of material needed for testing. ~~If pooling is used, it is recommended to pool organ pieces from a maximum of five fish.~~

4. Diagnostic methods

The methods currently available for ~~identifying infection~~ pathogen detection that can be used in i) surveillance of apparently healthy ~~populations~~ animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

~~The designations used in the Table indicate:~~

Ratings ~~against~~ for purposes of use. For each recommended assay a qualitative rating ~~against~~ for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, ~~successful application by diagnostic laboratories,~~ availability, cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

~~Key:~~

+++ =	~~Most suitable~~ Methods ~~—~~are most suitable with desirable performance and operational characteristics.
++ =	~~Suitable~~ Method~~(s)~~ are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	~~Less suitable~~ Methods ~~—~~are suitable, but performance or operational characteristics may ~~significantly~~ limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

~~The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.~~

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

OIE Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting

amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the OIE Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. OIE recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology					++	++	++	1				
Cytopathology												
Cell culture	+++	+++	+++	~~2~~ 1	+++	+++	+++	~~2~~ 1	~~++~~	~~++~~	~~++~~	~~2~~ 1
Immunohistochemistry					+	+	+	1				
Real-time PCR	+++	+++	+++	~~2~~ 1	+++	+++	+++	2	~~++~~	~~++~~	~~++~~	~~2~~ 1
Conventional PCR	+	+	+	1	++	++	++	1	~~++~~	~~++~~	~~++~~	~~1~~
Conventional PCR followed by amplicon sequencing									+++	+++	+++	~~3~~ 1
In-situ hybridisation												
Bioassy												
LAMP												
Ab-ELISA			+	1								
Ag-ELISA	+	+	+	1	+	+	+	1				
Other antigen detection methods[3]												
Other method[3]												

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

Light microscopy: routine methods can be used for tissue fixation, such as in 10% buffered neutral formalin, paraffin embedding, preparation of 4–10 μm sections and staining with H&E to demonstrate tissue necrosis and basophilic intracytoplasmic inclusion bodies. These inclusion bodies are indicative but not confirmatory for infection with EHNV. Formalin-fixed paraffin-embedded sections can also be stained using an immunoperoxidase method (see below) to identify EHNV antigen associated with necrotic lesions.

Acute focal, multifocal or locally extensive coagulative or liquefactive necrosis of liver, haematopoietic kidney and spleen are commonly seen in routine haematoxylin and eosin (H&E)-stained sections of formalin-fixed material. A small number of basophilic intracytoplasmic inclusion bodies may be seen, particularly in areas immediately surrounding necrotic areas in the liver and kidney. Necrotic lesions may also be seen in heart, pancreas, gastrointestinal tract, gill and pseudobranch (Reddacliff & Whittington, 1996).

Affected tissues (e.g. kidney, liver and spleen) contain cells exhibiting necrosis. Cells contain conspicuous cytoplasmic inclusions that are rarefied areas of the cytoplasm in which the viruses are assembled. ~~Within the cytoplasm, aggregates (paracrystalline arrays) of large (175 nm ± 6 nm) nonenveloped icosahedral viruses are apparent; single viruses are also present. Complete viruses (containing electron-dense cores) bud/egress from the infected cells through the plasma membrane.~~ The nuclei of infected cells are frequently located peripherally and are distorted in shape.

4.3. Cell culture for isolation

4.3.1. Preparation of fish tissues for virus isolation

A simple method for preparation of fish tissues for cell culture and ELISA has been validated (Whittington & Steiner, 1993) (see sampling Section 3).

i) Freeze tubes containing tissues at –80°C until needed.

ii) Add 0.5 ml of homogenising medium (minimal essential medium Eagle, with Earle's salts with glutamine] [MEM] with 200 International Units [IU] ml^{-1} penicillin, 200 μg ml^{-1} streptomycin and 4 μg ml^{-1} amphotericin B) to each tube. Grind tissue to a fine mulch with a sterile fitted pestle.

iii) Add another 0.5 ml of homogenising medium to each tube and mix with a pestle.

iv) Add three sterile glass beads to each tube (3 mm diameter) and close the lid of the tube.

v) Vortex the suspension vigorously for 20–30 seconds and place at 4°C for 2 hours.

vi) Vortex the suspension again as above and centrifuge for 10 minutes at 2500 ***g*** in a benchtop microcentrifuge.

vii) Transfer the supernatant, now called clarified tissue homogenate, to a fresh sterile tube. Homogenates may be frozen at –80°C until required for virus isolation and ELISA.

4.3.2. Cell culture~~/artificial media~~

EHNV ~~grows~~ replicates well in many fish cell lines including BF-2 (bluegill fry ATCC CCL 91), FHM (fathead minnow; ATCC CCL 42), EPC (*epithelioma papulosum cyprini* [Cinkova *et al.,* 2010]), and CHSE-214 (Chinook salmon embryo cell line; ATCC CRL 1681) at temperatures ranging from 15 to 22°C (Crane *et al.,* 2005). Incubation temperatures of 20°C or 24°C result in higher titres than 15°C; and BF-2, EPC, or CHSE 214 incubated at 22°C ~~and BF-2 EPC or CHSE-214 cells~~ are recommended to maximise titres, which might be important for the detection

of low numbers of viruses in fish tissues (Ariel *et al.,* 2009). BF-2 cells are preferred by the OIE Reference Laboratory with an incubation temperature of 22°C. The procedure for BF-2 cells is provided below. A procedure for CHSE-214 cells is provided under immunoperoxidase staining below (Section 4.7). The identity of viruses in cell culture is determined by ~~immunostaining, ELISA, immuno-electron microscopy,~~ PCR and amplicon sequencing.

4.3.3. Cell culture technical procedure

Samples: tissue homogenates.

Cells are cultured (in flasks, tubes or multi-well plates) with growth medium (MEM + 10% fetal ~~calf~~ bovine serum [F~~C~~BS] with 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 2 µg ml^{-1} amphotericin B). The cells are incubated until almost confluent at 22°C, which can take up to 4 days depending on the seeding rate. Medium is changed to a maintenance medium (MEM with 2% F~~C~~BS and 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 2 µg ml^{-1} amphotericin B) on the day of inoculation. A 1/10 dilution using homogenising medium is made of single or pooled homogenates. Each culture is inoculated with 100 µl of sample per ml of culture medium. This represents a final 1/100 dilution of a 0.1 mg ml^{-1} tissue homogenate. A further 1/10 dilution is made representing a final 1/1000 dilution, and two cultures are inoculated. No adsorption step is used. As an alternative, two to three cultures can be inoculated directly with 10 µl undiluted homogenate per ml of culture medium. Note that a high rate of cell toxicity or contamination often accompanies the use of a large undiluted inoculum. The cultures are incubated at 22°C in an incubator for 6 days. Cultures are read at days 3 and ~~day~~ 6. Cultures are passed at least once to detect samples with low levels of virus. On day 6, the primary cultures (P1) are frozen overnight at –20°C, thawed, gently mixed and then the culture supernatant is inoculated onto fresh cells as before (P2), i.e. 100 µl P1 supernatant per ml culture medium. Remaining P1 supernatants are transferred to sterile 5 ml tubes and placed at 4°C for testing by ELISA or PCR or another means to confirm the cause of cytopathic effect (CPE) as EHNV. P2 is incubated as above, and a third pass is conducted if necessary.

4.3.4. Interpretation of results

CPE is well developed and consists of focal lysis surrounded by rounded granular cells. This change extends rapidly to involve the entire monolayer, which detaches and disintegrates. Cell cultures can be tested for EHNV DNA using real-time PCR and conventional PCR with sequence analysis as described in Section 4.4. Antigen can be detected using immunocytochemistry in cell cultures with polyclonal antibodies and protocol available from the reference laboratory.

Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

4.4. Nucleic acid amplification

Although several conventional PCR or quantitative real-time PCR methods have been described for the detection of ranaviruses (Jaramillo *et al.,* 2012; Pallister *et al,* 2007; Stilwell *et al.,* 2018), EHNV can only be detected when these methods are combined with methods that specifically detect EHNV. ~~none has been adequately validated according to OIE guidelines for primary detection of EHNV. However, identification of ranavirus at genus and species level is possible using several published PCR strategies.~~

Samples can be screened by real-time PCR, but as the assays described are not specific for EHNV, identification of EHNV by conventional PCR and amplicon sequencing must be undertaken on any samples screening positive by real-time PCR. For testing by conventional PCR, two PCR assays using MCP primers are used with amplicon sequencing required to differentiate EHNV from ECV, FV3 and BIV (Marsh *et al*., 2002). ~~Alternatively, PCR of the DNA polymerase gene and neurofilament triplet H1-like protein genes can be used (Holopainen *et al*., 2011) (this method is not described in this chapter).~~

~~*Samples:* virus from cell culture or direct analysis of tissue homogenate.~~

PCR assays should always be run with the controls specified in Section 2.5 *Use of molecular techniques for surveillance testing, confirmatory testing and diagnosis* of Chapter 2.3.0 *General information* (diseases of fish). Each sample should be tested in duplicate.

Extraction of nucleic acids

Numerous different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid should be checked using optical density or by running a gel.

4.4.1. Real-time PCR

The ranavirus real-time screening protocol in use at the OIE Reference Laboratory is based on Pallister *et al.*, 2007. Alternative real-time PCR assays can be used according to published protocols for detection of the major capsid protein gene sequence of EHNV and other ranaviruses. The assay described by Jaramillo *et al.* (2012) uses SYBR Green detection chemistry and the assay described by Stilwell *et al.* (2018) detects multiple ranavirus species using hydrolysis probe detection chemistry.

~~Tissue samples can be homogenised by manual pestle grinding or by bead beating (Rimmer *et al.*, 2012). Commercially available nucleic acid extraction kits (e.g. spin columns, magnetic beads) may be used to extract DNA directly from tissues and from tissue homogenates and cell culture supernatants. Depending on the number of samples to be tested, in the OIE Reference Laboratory, nucleic acids are extracted with either the QIAamp Viral RNA Mini Kit (Qiagen) or MagMAX-96 Viral RNA Isolation Kit (Applied Biosystems) according to the manufacturer's instructions. A negative extraction control, consisting of extraction reagents only, is included when test samples are extracted.~~

~~The ranavirus real-time screening protocol in use at the OIE Reference Laboratory, based on Pallister *et al.*, 2007 is as follows; Template (2 µl) is added to 23 µl reaction mixture containing 12.5 µl TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM for each primer, 250 nM for probe, and molecular grade water. After 1 cycle of 50°C for 2 minutes and 95 °C for 10 minutes, PCR amplification consists of 45 cycles of 95°C for 15 seconds, 60°C for 60 seconds.~~

~~Alternative real-time PCR assays can be used according to published protocols for detection of the major capsid protein gene sequence of EHNV and other ranaviruses. The assay described by Jaramillo *et al.* (2012) uses SYBR Green detection chemistry and the assay described by Stilwell *et al.* (2018) was designed to detect multiple ranavirus species using hydrolysis probe detection chemistry.~~

~~The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.~~

~~***Table 4.4.1.1. Ranavirus primer and probe sequences***~~

~~Primer~~	~~Sequence (5'–3')~~	~~Reference~~
~~RANA CON F~~	~~5' CTC ATC GTT CTG GCC ATC A 3'~~	~~Pallister *et al.*, 2007~~
~~RANA CON R~~	~~5' TCC CAT CGA GCC GTT CA 3'~~	
~~**Probe**~~		
~~RANA CON Pr~~	~~5' 6**FAM** CAC AAC ATT ATC CGC ATC **MGB** 3'~~	
~~**Primer**~~		
~~C1096~~	~~GAC TGA CCA ACG CCA GCC TTA ACG~~	~~Jaramillo *et al.*, 2012~~
~~C1097~~	~~GCG GTG GTG TAC CCA GAG TTG TCG~~	
~~**Primer**~~		
~~RanaF1~~	~~CCA GCC TGG TGT ACG AAA ACA~~	~~Stilwell *et al.*, 2018~~
~~RanaR1~~	~~ACT GGG ATG GAG GTG GCA TA~~	
~~**Probe**~~		
~~RanaP1~~	~~6FAM TGG GAG TCG AGT ACT AC MGB~~	

Primer and probe sequences

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1 (Pallister *et al.*, 2007)			

Ranavirus	Fwd: RANA CON: CTC-ATC-GTT-CTG-GCC-ATC-A Rev: RANA CON: TCC-CAT-CGA-GCC-GTT-CA Probe: RANA CON Pr FAM-CAC-AAC-ATT-ATC-CGC-ATC-MGB	900 nM for each primer, 250 nM for probe	45 cycles of 95°C/15 sec; 60°C/60 sec

The ranavirus real-time screening protocol in use at the OIE Reference Laboratory, based on Pallister *et al.*, 2007. Alternative real-time PCR assays can be used according to published protocols for detection of the major capsid protein gene sequence of EHNV and other ranaviruses. The assay described by Jaramillo *et al.* (2012) uses SYBR Green detection chemistry and the assay described by Stilwell *et al.* (2018) detects multiple ranavirus species using hydrolysis probe detection chemistry.

Details of the controls to be run with each assay are set out in Section 5.5. of Chapter 2.2.1. of Section 2.2.

4.4.2. Conventional PCR

PCR and restriction endonuclease analysis (REA): technical procedure

Amplified product from PCR assay MCP-1 digested with PflM I enables differentiation of EHNV and BIV from FV3 and ECV. Amplified product from PCR assay MCP-2 digested with Hinc II, Acc I and Fnu4H I (individually) enables differentiation of EHNV and BIV from each other and from FV3 and ECV.

Preparation of reagents

EHNV purified DNA and BIV purified DNA PCR control reagents are supplied by the reference laboratory in freeze-dried form. Reconstitute using 0.5 ml of Tris-EDTA (TE) buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. For routine use, as a PCR control, it is recommended that working stocks be prepared as a 1/10 dilution in TE buffer (pH 8.0). Aliquots of 250 µl should be stored at −20°C. Each aliquot is sufficient for at least 50 reactions (1 to 5 µl added to cocktail) and has a minimum shelf life of 6 months from date of diluting.

Primers M151 and M152 (MCP-1, 321 bp), M153 and M154 (MCP-2, 625 bp) are supplied in working strength (100 ng μl^{-1}) and should be stored at −20°C. Primers can also be ordered from commercial suppliers. For primer sequences, refer to Table 4.4.2.1.

Table 4.4.2.1. MCP-1 and MCP-2 primer sequences

PCR assay	Primer	Sequence (5'–3')	Product size	Gene location
MCP-1	M151	AAC-CCG-GCT-TTC-GGG-CAG-CA	321 bp	266–586
	M152	CGG-GGC-GGG-GTT-GAT-GAG-AT		
MCP-2	M153	ATG-ACC-GTC-GCC-CTC-ATC-AC	625 bp	842–1466
	M154	CCA-TCG-AGC-CGT-TCA-TGA-TG		

PCR cocktail

Amplification reactions in a final volume of 50 µl (including 5 µl DNA sample) contain 2.5 µl (250 ng) of each working primer, 200 µM of each of the nucleotides dATP, dTTP, dGTP and dCTP, 5 µl of 10 × PCR buffer (66.6 mM Tris/HCl, 16.6 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 1.65 mg ml^{-1} BSA, 10 mM beta-mercaptoethanol) and 2 U Taq polymerase. Instructions on preparation of 10 × PCR buffer are included in Table 4.4.2.2.

Table 4.4.2.2. 10 × PCR buffer preparation

Ingredients	Amount	Final concentration in 50 µl PCR mix
Tris	4.050 g	66.6 mM
Ammonium sulphate	1.100 g	16.6 mM

Ingredients	Amount	Final concentration in 50 µl PCR mix
BSA (albumin bovine fraction V fatty acid free)	0.825 g	1.65 mg ml^{-1}
Magnesium chloride	1.25 ml	2.5 mM
TE buffer (sterile)	50 ml	

NOTE: alternative commercial buffers may also be used.

Two negative controls are included, one comprising PCR cocktail only and the second containing 5 µl TE buffer.

The MCP-1 and MCP-2 reactions have the following profile: 1 cycle of denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 1 minute; a final extension of 72°C for 5 minutes, and cooling to 4°C.

NOTE: the annealing temperature may be increased to 60 or 62°C to reduce nonspecific amplification when the assay is used to test fish tissues.

PCR results are assessed by electrophoresis in 2% agarose gels stained with ethidium bromide. EHNV PCR control DNA (1/10 working stock) should give a result similar in intensity to the 10–3 band in both cases.

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

Primer and probe sequences

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Product size MCP-1 is 321 bp and product size MCP-2 is 625 bp			
MCP-1 Gene location: 266-586 MCP-2 Gene location: 842-1466	M151: AAC-CCG-GCT-TTC-GGG-CAG-CA M152: CGG-GGC-GGG-GTT-GAT-GAG-AT M153: ATG-ACC-GTC-GCC-CTC-ATC-AC MI54: CCA-TCG-AGC-CGT-TCA-TGA-TG	250 ng of each primer	35 cycles of 50°C for 30 sec NOTE: the annealing temperature may be increased to 60 or 62°C to reduce non-specific amplification when the assay is used to test fish tissues.

4.4.3. Other nucleic acid amplification methods

Not applicable.

4.5. Amplicon sequencing

Amplicons generated using the MCP-1 and/or MCP-2 primers sets can be sequenced. Amplicons should be gel-purified and sequenced using both the forward and reverse primer. Consensus sequence, generated after analysis of the quality of the sequence chromatograms, can then be compared to reference sequences, for example by BlastN search of the NCBI database.

The size of the PCR amplicon is verified by agarose gel electrophoresis, and purified by excision from this gel. Both DNA strands must be sequenced and analysed and compared with published sequences.

4.6. *In-situ* hybridisation

Not applicable

4.7. Immunohistochemistry

Immunohistochemistry (immunoperoxidase stain)

Samples: formalin-fixed paraffin-embedded tissue sections.

Technical procedure

The following protocol is intended for the qualitative demonstration of EHNV antigens in formalin-fixed paraffin-embedded tissue sections (Reddacliff & Whittington, 1996). It assumes that antigens may have become cross linked and therefore includes a protease digestion step that may be omitted if unfixed samples are examined. A commercial kit (DAKO® LSAB K0679) with peroxidase-labelled streptavidin and a mixture of biotinylated anti-rabbit/anti-mouse/anti-goat immunoglobulins as link antibodies is used for staining. Other commercially supplied reagents are also used. For convenience these are also supplied by DAKO [15]. The primary affinity purified ~~rabbit~~ anti-EHNV antibody (Lot No. M708) is supplied freeze-dried by the OIE Reference Laboratory.

i) Cut 5 μm sections and mount on SuperFrost® Plus G/Edge slides (Menzel-Glaser, HD Scientific Cat. No. HD 041300 72P3). Mark around the section with a diamond pencil to limit the spread of reagents.

ii) Deparaffinise the section:

Preheat slides in a 60°C incubator for 30 minutes.

Place slides in a xylene bath and incubate for 5 minutes. Repeat once. Note that xylene replacements can be used without deleterious effects.

Tap off excess liquid and place slides in absolute ethanol for 3 minutes. Repeat once.

Tap off excess liquid and place slides in 95% ethanol for 3 minutes. Repeat once.

Tap off excess liquid and place slides in distilled or deionised water for 30 seconds.

iii) Expose antigens using a protease treatment. Flood slide with proteinase K (5–7 μg ml^{-1}) and incubate for 20 minutes (ready-to-use solution, DakoCytomation Cat. No. S3020). Rinse slide by immersing three times in water. Place in a PBST bath for 5 minutes (PBS pH 7.2, 0.05% [v/v] Tween 20). Tap off the excess wash solution and carefully wipe around the section.

iv) Perform the immunostaining reaction using the Universal DAKO LSAB®+ Kit, Peroxidase (DakoCytomation Cat No. K0679). Ensuring the tissue section is completely covered, add the following reagents to the slide. Avoid drying out.

v) 3% hydrogen peroxide: cover the section and incubate for 5 minutes. Rinse gently with PBST and place in a fresh wash bath.

vi) Primary antibody (affinity purified rabbit anti-EHNV 1:/1500 Lot No. M708) and negative control reagent (non-immune rabbit serum at a dilution of 1/1500) on a second slide. Cover the section and incubate for 15 minutes. Rinse slides.

vii) Biotin-labelled secondary link antibody: ~~Link:~~ cover the section and incubate for 15 minutes. Rinse slides.

viii) Streptavidin peroxidase: cover the section and incubate for 15 minutes. Rinse slides.

ix) Substrate–chromogen solution: cover the section and incubate for 5 minutes. Rinse slides gently with distilled water.

x) Counterstain by placing slides in a bath of DAKO® Mayer's Haematoxylin for 1 minute (Lillie's Modification, Cat. No. S3309). Rinse gently with distilled water. Immerse 10 times into a water bath. Place in distilled or deionised water for 2 minutes.

xi) Mount and cover-slip samples with an aqueous-based mounting medium (DAKO® Faramount Aqueous Mounting Medium Cat. No. S3025).

Interpretation of results

EHNV antigen appears as a brown stain in the areas surrounding degenerate and necrotic areas in parenchymal areas. There should be no staining with negative control rabbit serum on the same section.

15 Dako Cytomation California Inc., 6392 via Real, Carpinteria, CA 93013, USA, Tel.: (+1-805) 566 6655, Fax: (+1-805) 566 6688; Dako Cytomation Pty Ltd, Unit 4, 13 Lord Street, Botany, NSW 2019, Australia, Fax: (+61-2) 9316 4773; Visit http://www.dakosytomahon.com for links to other countries.

Availability of test and reagents: antibody reagents and test protocols are available from the OIE Reference Laboratory.

4.8. Bioassay

Not applicable.

4.9. Antibody- or antigen-based detection methods

An antigen ELISA for detection of EHNV and an EHNV antibody detection ELISA have been described (Whittington & Steiner, 1993). The same antibodies are suitable for immunohistochemistry on fixed tissues and for detection of ranavirus antigen in cell culture. Reagents and protocols are available from the reference laboratory. It should be noted that polyclonal antibodies used in all related methods (immunoperoxidase, antigen-capture ELISA and immunoelectron microscopy) cross-react with all known ranaviruses except Santee Cooper ranaviruses (Ahne *et al.,* 1998; Cinkova *et al.,* 2010; Hedrick *et al*., 1992; Hyatt *et al.*, 2000).

4.10. Other methods

Neutralising antibodies have not been detected in fish or mammals exposed to EHNV. Indirect ELISA for detection of antibodies induced following exposure to EHNV has been described for rainbow trout and European perch (Whittington *et al*., 1994; 1999; Whittington & Reddacliff, 1995). The sensitivity and specificity of these assays in relation to a standard test are not known and interpretation of results is difficult. Protocols and specific anti-immunoglobulin reagents required to conduct these tests are available from the reference laboratory.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR is the most appropriate method of screening healthy fish populations for EHNV; however, the available methods are not specific for EHNV. Any real-time PCR positive samples should be tested by conventional PCR and sequence analysis to distinguish EHNV from other ranaviruses.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory, and if necessary, refer samples to that laboratory for testing.

6.1. Apparently healthy animals or animals of unknown health status [16]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link to an infected population. ~~Geographic~~ Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with EHNV shall be suspected if at least one of the following criteria is met:

i) EHNV-typical CPE in cell culture ~~Positive result for EHNV based on virus isolation in cell cultures~~

[16] For example transboundary commodities.

ii) Positive real-time or conventional PCR result

iii) Positive EHNV antigen ELISA

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with EHNV is considered to be confirmed if at least one of the following criteria is met:

i) EHNV-typical CPE in cell culture followed by identification of EHNV by conventional PCR and sequence analysis of the amplicon;

ii) A positive result in tissue samples by real-time PCR and identification of EHNV by conventional PCR and sequence analysis of the amplicon.

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with EHNV shall be suspected if at least one of the following criteria is met:

i) Histopathology consistent with EHNV;

ii) EHNV-typical CPE in cell cultures;

iii) Positive real-time or conventional PCR result.

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with EHNV is considered to be confirmed if, in addition to the criteria in Section 6.2.1, at least one of the following criteria is met:

i) EHNV-typical CPE in cell culture followed by identification of EHNV by conventional PCR and sequence analysis of the amplicon;

ii) A positive result in tissue samples by real-time PCR and identification of EHNV by conventional PCR and sequence analysis of the amplicon.

~~Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with EHNV are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with EHNV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Clinically diseased fish (multiple species) from disease outbreaks and experimental infections	Pool of kidney, liver and spleen from individual fish	European perch (*Perca fluviatilis*), river blackfish (*Gadopsis marmoratus*), golden perch (*Macquaria ambigua*), trout cod (*Maccullochella macquariensis*), freshwater catfish (*Tandanus tandanus*), Macquarie perch (*Macquaria australasica*) rainbow trout (*Oncorhynchus mykiss*)	94.3%* (n= 105)	100% (n= 441)	Virus isolation in BF-2 cell culture	Jaramillo *et al.,* (2012)
Real-time PCR	Diagnosis	Clinically diseased fish (multiple species) from disease outbreaks and experimental infections	Pool of kidney, liver and spleen from individual fish	European perch (*Perca fluviatilis*), river blackfish (*Gadopsis marmoratus*), golden perch (*Macquaria ambigua*), trout cod (*Maccullochella macquariensis*), freshwater catfish (*Tandanus tandanus*), Macquarie perch (*Macquaria australasica*) rainbow trout (*Oncorhynchus mykiss*)	95%* (n=106)	100% (n=80)	Virus isolation in BF-2 cell culture	Stilwell *et al.,* 2018

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study;
PCR: = polymerase chain reaction. Note: these assays detect multiple ranaviruses in addition to EHNV that infect amphibian hosts. *A positive result requires characterisation using sequencing to confirm that the result indicates the presence of EHNV.

6.3.2. For surveillance of apparently healthy animals: not available

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe: = diagnostic sensitivity, DSp = diagnostic specificity, qPCR: = real-time polymerase chain reaction.

7. References

AHNE W., BEARZOTTI M., BREMONT M. & ESSBAUER S. (1998). Comparison of European systemic piscine and amphibian iridoviruses with epizootic haematopoietic necrosis virus and frog virus 3. *J. Vet. Med.* [*B*], **45**, 373–383.

AHNE W., OGAWA M. & SCHLOTFELDT H.J. (1990). Fish viruses: transmission and pathogenicity of an icosahedral cytoplasmic deoxyribovirus isolated from sheatfish *Silurus glanis*. *J. Vet. Med*. [*B*], **37**, 187–190.

AHNE W., SCHLOTFELDT H.J. & THOMSEN I. (1989). Fish viruses: isolation of an icosahedral cytoplasmic deoxyribovirus from sheatfish (*Silurus glanis*). *J. Vet. Med.* [*B*], **36**, 333–336.

ARIEL E. & BANG JENSEN B. (2009). Challenge studies of European stocks of redfin perch, *Perca fluviatilis* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), with epizootic haematopoietic necrosis virus. *J. Fish Dis.,* **32**, 1017–1025.

Ariel E, Holopainen R, Olenen NJ & Tapiovaara H (2010). Comparative study of ranavirus isolates from cod (*Gadua morhua)* and turbot (*Psetta maxima*) wuth reference to other ranaviruses. Archives of Virology **155**, 1261-1271

ARIEL E., NICOLAJSEN N., CHRISTOPHERSEN M.-B., HOLOPAINEN R., TAPIOVAARA H. & BANG JENSEN B. (2009). Propagation and isolation of ranaviruses in cell culture. *Aquaculture,* **294**, 159–164.

BECKER J.A., TWEEDIE A., GILLIGAN D., ASMUS M. & WHITTINGTON R. J. (2016). Susceptibility of Australian Redfin Perch *Perca fluviatilis* Experimentally Challenged with Epizootic Hematopoietic Necrosis Virus (EHNV). *J. Aquat. Anim. Health*, **28**, 122–130.

BLOCH B. & LARSEN J.L. (1993). An iridovirus-like agent associated with systemic infection in cultured turbot *Scophthalmus maximus* fry in Denmark. *Dis. Aquat. Org*., **15**, 235–240.

BRYAN L.K., BALDWIN C.A., GRAY M.J. & MILLER D.L. (2009). Efficacy of select disinfectants at inactivating Ranavirus. *Dis. Aquat. Org.,* **84**, 89–94.

CHINCHAR V.G. (2002). Ranaviruses (family Iridoviridae): emerging cold-blooded killers – brief review. *Arch. Virol.*, **147**, 447–470.

CHINCHAR G., ESSBAUER S., HE J.G., HYATT A., MIYAZAKI T., SELIGY V. & WILLIAMS T. (2005). Family Iridoviridae. *In:* Virus Taxonomy. Classification and Nomeclature of Viruses. Eight Report of the International Committee on the Taxonomy of Viruses, Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U. & Ball L.A., eds. Academic Press, San Diego, California, USA, 145–161.

CINKOVA K., RESCHOVA S., KULICH P. & VESELY T. (2010). Evaluation of a polyclonal antibody for the detection and identification of ranaviruses from freshwater fish and amphibians. *Dis. Aquat. Org.,* **89**, 191–198.

CRANE M.S.J., YOUNG J. & WILLIAMS L. (2005). Epizootic haematopoietic necrosis virus (EHNV): growth in fish cell lines at different temperatures. *Bull. Eur. Assoc. Fish Pathol*., **25**, 228–231.

DRURY S.E.N., GOUGH R.E. & CALVERT I. (2002). Detection and isolation of an iridovirus from chameleons (*Chamaeleo quadricornis* and *Chamaeleo hoehnelli*) in the United Kingdom. *Vet. Rec.,* **150**, 451–452.

FIJAN N., MATASIN Z., PETRINEC Z., VALPOTIC I. & ZWILLENBERG L.O. (1991). Isolation of an iridovirus-like agent from the green frog (*Rana esculenta* L.). *Veterinarski Arhiv,* **61**, 151–158.

HEDRICK R.P., MCDOWELL T.S., AHNE W., TORHY C. & DE KINKELIN P. (1992). Properties of three iridovirus-like agents associated with systemic infections of fish. *Dis. Aquat. Org.,* **13**, 203–209.

~~HOLOPAINEN R., HONKANEN J., JENSEN B.B., ARIEL E. & TAPIOVAARA H. (2011). Quantitation of ranaviruses in cell culture and tissue samples. *J. Virol. Methods,* **171**, 225–233.~~

HOLOPAINEN R., OHLEMEYER S., SCHÜTZE H., BERGMANN S.M. & TAPIOVAARA H. (2009). Ranavirus phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofilament triplet H1-like protein genes. *Dis. Aquat. Org.,* **85**, 81–91.

HYATT A.D., GOULD A.R., ZUPANOVIC Z., CUNNINGHAM A.A., HENGSTBERGER S., WHITTINGTON R.J., KATTENBELT J. & COUPAR B.E.H. (2000). Comparative studies of piscine and amphibian iridoviruses. *Arch. Virol.,* **145**, 301–331.

HYATT A.D., WILLIAMSON M., COUPAR B.E.H., MIDDLETON D., HENGSTBERGER S.G., GOULD A.R., SELLECK P., WISE T.G., KATTENBELT J., CUNNINGHAM A.A.& LEE J. (2002). First identification of a ranavirus from green pythons (*Chondropython viridis*). *J. Wildl. Dis.,* **38**, 239–252.

JARAMILLO D., TWEEDIE A., BECKER J.A., HYATT A., CRAMERI S. & WHITTINGTON R.J. (2012). A validated quantitative polymerase chain reaction assay for the detection of ranaviruses (Family Iridoviridae) in fish tissue and cell cultures, using EHNV as a model. *Aquaculture*, **356–357**, 186–192.

LANGDON J.S. (1989). Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, *Perca fluviatilis* L., and 11 other teleosts. *J. Fish Dis.*, **12**, 295–310.

LANGDON J.S. & HUMPHREY J.D. (1987). Epizootic Hematopoietic Necrosis a New Viral Disease in Redfin Perch *Perca fluviatilis* L. in Australia. *J. Fish Dis.*, **10**, 289–298.

LANGDON J.S., HUMPHREY J.D. & WILLIAMS L.M. (1988). Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, *Salmo gairdneri* Richardson, in Australia. *J. Fish Dis.*, **11**, 93–96.

LANGDON J.S., HUMPHREY J.D., WILLIAMS L.M., HYATT A.D. & WESTBURY H.A. (1986). First virus isolation from Australian fish: an iridovirus-like pathogen from redfin perch, *Perca fluviatilis* L. *J. Fish Dis.*, **9**, 263–268.

MAO J., THAM T.N., GENTRY G.A., AUBERTIN A. & CHINCHAR V.G. (1996). Cloning, sequence analysis, and expression of the major capsid protein of the iridovirus frog virus 3. *Virology*, **216**, 431–436.

MAO J.H., HEDRICK R.P. & CHINCHAR V.G. (1997). Molecular characterisation, sequence analysis and taxonomic position of newly isolated fish iridoviruses. *Virology*, **229**, 212–220.

MARSH I.B., WHITTINGTON R.J., O'ROURKE B., HYATT A.D. & CHISHOLM O. (2002). Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Molec. Cell. Probes*, **16**, 137–151.

PALLISTER J., GOULD A., HARRISON D., HYATT A., JANCOVICH J. & HEINE H. (2007). Development of real-time PCR assays for the detection and differentiation of Australian and European ranaviruses. *J. Fish Dis.,* **30**, 427–438.

POZET F., MORAND M., MOUSSA A., TORHY C. & DE KINKELIN P. (1992). Isolation and preliminary characterization of a pathogenic icosahedral deoxyribovirus from the catfish (*Ictalurus melas*). *Dis. Aquat. Org.,* **14**, 35–42.

REDDACLIFF L.A. & WHITTINGTON R.J. (1996). Pathology of epizootic haematopoeitic necrosis virus (EHNV) infection in rainbow trout (*Oncorhynchus mykiss* Walbaum) and redfin perch (*Perca fluviatilis* L.). *J. Comp. Pathol.,* **115**, 103–115.

RIMMER A.E., BECKER J.A., TWEEDIE A. & WHITTINGTON R.J. (2012). Validation of high throughput methods for tissue disruption and nucleic acid extraction for ranaviruses (family Iridoviridae). *Aquaculture*, **338–341**, 23–28.

SPEARE R. & SMITH J.R. (1992). An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. *Dis. Aquat. Org.*, **14**, 51–57.

STILWELL N.K., WHITTINGTON R.J., HICK P.M., BECKER J.A., ARIEL E., VAN BEURDEN S., VENDRAMIN N., OLESEN N.J. & WALTZEKT.B. (2018). Partial validation of a TaqMan real-time quantitative PCR for the detection of ranaviruses. *Dis. Aquat. Org.*, **128**, 105–116.

WHITTINGTON R.J., BECKER J.A. & DENNIS M.M. (2010). Iridovirus infections in finfish – critical review with emphasis on ranaviruses. *J. Fish Dis.,* **33**, 95–122.

WHITTINGTON R.J., KEARNS C., HYATT A.D., HENGSTBERGER S. & RUTZOU T. (1996). Spread of epizootic haematopoietic necrosis virus (EHNV) in redfin perch (*Perca fluviatilis*) in southern Australia. *Aust. Vet. J.*, **73**, 112–114.

WHITTINGTON R.J., PHILBEY A., REDDACLIFF G.L. & MACGOWN A.R. (1994). Epidemiology of epizootic haematopoietic necrosis virus (EHNV) infection in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum): findings based on virus isolation, antigen capture ELISA and serology. *J. Fish Dis.*, **17**, 205–218.

WHITTINGTON R.J. & REDDACLIFF G.L. (1995). Influence of environmental temperature on experimental infection of redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) with epizootic haematopoietic necrosis virus, an Australian iridovirus. *Aust. Vet. J.,* **72**, 421–424.

WHITTINGTON R.J., REDDACLIFF L.A., MARSH I., KEARNS C., ZUPANOVIC Z. & CALLINAN R.B. (1999). Further observations on the epidemiology and spread of epizootic haematopoietic necrosis virus (EHNV) in farmed rainbow trout *Oncorhynchus mykiss* in southeastern Australia and a recommended sampling strategy for surveillance. *Dis. Aquat. Org.,* **35**, 125–130.

WHITTINGTON R.J. & STEINER K.A. (1993). Epizootic haematopoietic necrosis virus (EHNV): improved ELISA for detection in fish tissues and cell cultures and an efficient method for release of antigen from tissues. *J. Virol. Methods,* **43**, 205–220.

WOLF K., BULLOCK G.L., DUNBAR C.E. & QUIMBY M.C. (1968). Tadpole edema virus: a viscerotrophic pathogen for anuran amphibians. *J. Infect. Dis.*, **118**, 253–262.

ZUPANOVIC Z., MUSSO C., LOPEZ G., LOURIERO C.L., HYATT A.D., HENGSTBERGER S. & ROBINSON A.J. (1998). Isolation and characterisation of iridoviruses from the giant toad *Bufo marinus* in Venezuela. *Dis. Aquat. Org.,* **33**, 1–9.

*
* *

NB: There is an OIE Reference Laboratory for infection with epizootic haematopoietic necrosis virus (EHNV) (please consult the OIE web site for the most up-to-date list: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3). Please contact the OIE Reference Laboratories for any further information on infection with EHNV. The OIE Reference Laboratory can supply purified EHNV DNA, heat killed EHNV antigen and polyclonal antibodies against EHNV together with technical methods. A fee is charged for the reagents to cover the costs of operating the laboratory.

NB: FIRST ADOPTED IN 1995 AS EPIZOOTIC HAEMATOPOIETIC NECROSIS; MOST RECENT UPDATES ADOPTED IN 2018.

Annexe 27. Point 7.2.3. – Section 2.2.1. du chapitre 2.3.9. « Infection par le virus de la virémie printanière de la carpe »

CHAPTER 2.3.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

[…]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with SVCV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Cyprinidae	*Abramis brama*	Bream
	Aristichthys nobilis	Bighead carp
	Carassius auratus	Goldfish
	Ctenopharyngodon idella	Grass carp
	Cyprinus carpio	Common carp (all varieties and subspecies)
	Danio rerio	Zebrafish
	Notemigonus crysoleucas	Golden shiner
	Pimephales promelas	Fathead minnow
	Percocypris pingi	Jinsha bass carp
	Rutilus kutum	Caspian white fish
	Rutilus rutilus	Roach
Siluridae	*Silurus glanis*	Wels catfish

[…]

Annexe 28. Point 7.3.1. – Sections 2.2.1. et 2.2.2. du chapitre 2.4.2. « Infection à *Bonamia exitiosa* »

CHAPTER 2.4.2.

INFECTION WITH *BONAMIA EXITIOSA*

[...]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Bonamia exitiosa* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: Argentinean flat oyster (*Ostrea puelchana*), Australian mud oyster (*Ostrea angasi*), Chilean flat oyster (*Ostrea chilensis*), crested oyster (*Ostrea equestris*), eastern oyster (*Crassostrea virginica*), European flat oyster (*Ostrea edulis*), Olympia oyster (*Ostrea lurida*) and Suminoe oyster (*Magallana* (syn. *Crassostrea*) *ariakensis*).

2.2.1. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *B. exitiosa* according to Chapter 1.5 of the *Aquatic Code* are: dwarf oyster (*Ostrea stentina*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: Pacific cupped oyster (*Magallana* [syn. *Crassostrea*] *gigas*) and Sydney rock oyster (*Saccostrea glomerata*).

[...]

CHAPTER 2.4.3.

INFECTION WITH *BONAMIA OSTREAE*

[. . .]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Bonamia ostrea* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: European flat oyster (*Ostrea edulis*), Chilean flat oyster (*Ostrea chilensis*), and Suminoe oyster (*Magallana* [syn. *Crassostrea]* *ariakensis*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *B. ostreae* according to Chapter 1.5 of the *Aquatic Code* are: Argentinean flat oyster (*Ostrea puelchana*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: beadlet anemone (*Actina equina*), brittle star (*Ophiothrix fragilis*), European sea squirt (*Ascidiella aspersa*), grouped zooplankton and Pacific cupped oyster (*Magallana* [syn. *Crassostrea*] *gigas*).

[...]

Annexe 30. Point 7.3.2. – Sections 2.2.1. et 2.2.2. du chapitre 2.4.4. « Infection à *Marteilia refringens* »

CHAPTER 2.4.4.

INFECTION WITH *MARTEILIA REFRINGENS*

[...]

2.2. Host factors

2.2.1. Susceptible host species

~~*Oyster species: Ostrea edulis* (Grizel *et al.*, 1974); and mussel species: *Mytilus* species including *M. edulis* (Le Roux *et al.*, 2001) and *M. galloprovincialis* (López-Flores *et al.*, 2004; Novoa *et al.*, 2005; Robledo *et al.*, 1995a; Villalba *et al.*, 1993b).~~

~~Infection with *M. refringens* was demonstrated in the oyster *Ostrea stentina*, the clam species *Solen marginatus* (López-Flores *et al.*, 2008a) and *Chamelea gallina* (López-Flores *et al.*, 2008b) and the mussel *Xenostrobus securis* (Pascual *et al.*, 2010).~~

~~Other *Ostrea* species including *O. chilensis, O. puelchana, O. angasi*, and *O. denselamellosa* were found to be infected with *Marteilia* sp. when deployed in an infected area (Berthe *et al.*, 2004; Martin, 1993). However, in these cases, the parasite identification was not done at the molecular level.~~

~~In addition, different stages, including mature stages, of parasites looking like *M. refringens*, were observed by histology in cockles (*Cerastoderma edule*), clam species (*Ruditapes decussatus R. philippinarum*, *Tapes rhomboides*, *T. pullastra*, *Ensis minor, E. siliqua*), and oysters (*Crassostrea virginica*) among other bivalve species (Berthe *et al.*, 2004; López-Flores *et al.*, 2008b). In all these cases, parasite identification is uncertain.~~

~~Lastly, the copepod *Paracartia grani* was shown to be susceptible to *M. refringens* and this species could participate in the transmission of the parasites between bivalves (see 2.3.1)~~

Species that fulfil the criteria for listing as susceptible to infection with *Marteilia refringens* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: blue mussel (*Mytilus edulis*), dwarf oyster (*Ostrea stentina*), European flat oyster (*Ostrea edulis*), European razor clam (*Solen marginatus*), golden mussel (*Xenostrobus securis*), Mediterranean mussel (*Mytilus galloprovincialis*) and striped venus (*Chamelea gallina*).

Additionally, a copepod species (*Paracartia grani*) has been found to meet the criteria for listing as susceptible to infection with *Marteilia refringens* and is considered an intermediate host.

2.2.2. ~~Susceptible stages of the host~~ Species with incomplete evidence for susceptibility

~~Juveniles and older life stages are known to be susceptible (Grizel, 1985).~~

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *M.refringens* according to Chapter 1.5. of the *Aquatic Code* are: Chilean flat oyster (*Ostrea chilensis*), a copepod (*Paracartia latisetosa*) and Japanese flat oyster (*Ostrea denselamellosa*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: Cortez oyster (*Crassostrea corteziensis*), grooved carpet shell (*Ruditapes decussatus*), Pacific cupped oyster (*Magallana* [syn. *Crassostrea] gigas*) and zooplankton (*Acartia discaudata*, *Centropages typicus*, *Euterpina acutifrons*, unidentified *Oithona sp.*, *Penilia avirostris*).

[...]