OIE Collaborating Centres Reports Activities

Activities in 2021

This report has been submitted : 2022-01-21 10:54:01

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ToR: To provide services to the OIE, in particular within the region, in the designated specialty, in support of the implementation of OIE policies and, where required, seek for collaboration with OIE Reference Laboratories

ToR: To identify and maintain existing expertise, in particular within its region

1. Activities as a centre of research, expertise, standardisation and dissemination of techniques within the remit of the mandate given by the OIE

Epidemiology, surveillance, risk assessment, modelling	
Title of activity	**Scope**
Epidemiology study on canine leptospirosis in north-eastern Italy	Identification and territorial diffusion of Leptospira serovars responsible for the disease in dogs; evaluation of climatic-environmental factors (temperature, rainfall, geographical area) influencing the risk of infection; adequacy of vaccines currently available for dogs; risk of infection in humans in categories of "exposed" and "non-exposed" subjects based on serological studies. (research project, IZSVE RC 05/17). n. 75 DNA/strains were submitted to the IZSLER National Reference Centre for Leptospirosis in 2021. Epidemiological data presented in the final report of the IZSVE RC 05/17.
Zoonoses in shelters for dogs and cats: study and development of an integrated strategy (epidemiology, social research, training and risk communication) for effective health management	Survey on known and potential zoonoses in dogs and cat in shelters (Leptospira spp., Brucella canis, Leishmania infantum, dermatofitosis, antibiotic-resistant bacteria, Capnocytophaga canimorsus, Bartonella henselae, Norovirus, Rotavirus, Cowpox virus, Mammalian Orthoreovirus, Hepatitis E, SARS-CoV-2); n. 157 dogs and 197 cats collected in 2021. Social research presented in the medium term report of IZSVE RC 12/19.

Monitoring the focus of Echinococcus multilocularis in red fox	To infer the correlation between fox density and Echinococcus multilocularis occurrence, 115 faecal samples were collected and subsequently genotyped at 21 microsatellite loci in collaboration with Edmund Mach Foundation (FEM). The resulting genotypes (n = 31 for Echinococcus multilocularis positive area of Alto Isarco district; n= 25 for Echinococcus multilocularis negative area of Ultimo valley) were used to conduct a spatially explicit capture-recapture analysis, which allowed to infer fox population densities at 2.11 ± 62.78 individuals/km2 in Alto Isarco, and 7.51 ± 2 ind/km2 in Ultimo valley, thus indicating that no direct correlation exists between fox population density and the presence of Echinococcus multilocularis foci. In 2021, after the one positive red fox found in 2020 in Trento province (Fassa valley), the surveillance strategy was intensified in the area, analyzing 19 fox carcasses. Samplings were carried out in collaboration with the Forest and Fauna department of Province Trento and Science Museum, after all collaborating personnel was sensitized on safety sampling procedures. Intestinal scraping technique were implemented. No further Echinococcus multilocularis positive cases were found.

Monitoring the focus of Echinococcus multilocularis in small rodents	In 2020/21, the Centre offered a diagnostic service for the search of Echinococcus multilocularis in small rodents (voles) on the area of competence. With collaboration of Museo delle Scienze di Bolzano, 25 dead voles were delivered to the IZSVe Laboratory. At the necroscopy, liver was picked up and tested with PCR multilocus. 4 sampling tested positive (16%). Three of the positive samples come from areas with a constant and confirmed presence of Echinococcus multilocularis, while one in particular that coming from the Alta Val Venosta, was collected in an area where some historical positives were reported, subsequently no longer confirmed.
Diagnosis of Hantavirus in small rodents	In spring-summer 2021, in the frame of the cyclic fluctuations of small rodents, a mortality outbreak occurred across North Eastern Italy. Molecular investigations were performed on pools of rodents from different locations and, for the first time in Italy, a virological positivity for Hantavirus was observed, namely in wild yellow-necked mice (Apodemus flavicollis) from the Tarvisio area, close to the border with Slovenia. The genetic characterization classified the isolates as Dobrava virus. Dobrava viruses were identified for the first time in 1992 in Slovenia and, just to mention the areas closest to Italy, they are present all across the Balkans and in Central-Eastern Europe, where they have mice of the genus Apodemus as their main reservoir hosts. Their detection in the area bordering Slovenia suggests a possible expansion of the range of these pathogens into Northeast Italy. At the same time, the negativity of the samples from Veneto and Trentino, where outbreaks were also evident, indicates that the presence of Hantavirus is not directly related to rodent cyclic fluctuations.

Entomological surveillance for West Nile and Usutu virus in northeastern Italy	In 2021, we placed 77 CDC-CO2 mosquito traps over the area and collected 121947 mosquitoes of 18 different species. The viral search was done in 2591 pooled specimens. West Nile virus was detected in 17 pools of Culex pipiens, and 1 pool both of Ochlerotatus caspius and Aedes albopictus.USUTU virus was found in 43 pools of Cx. pipiens and 1 pool of Oc. caspius, respectively.
Surveillance of Invasive mosquitoes in “Points of entry”	We monitored the entry and spread of invasive species of mosquitoes of the genus Aedes, through the surveillance of certain "Points of entry" such as the port of Marghera (Venice) and the airport of Venice and Treviso (northeast Italy). Aedes albopictus species have been collected in all sites, while no other invasive species were found.
Surveillance of Aedes japonicus japonicus and Ae. koreicus in Italy	The invasive mosquito species, Aedes koreicus, and Ae. japonicus japonicus was detected in northeastern Italy for the first time in 2011 and 2015 respectively. Active monitoring has been carried out since their introduction to assess the spreading and occurrence of these species. The presence of invasive mosquitoes was checked in all possible breeding sites through collections of larvae. The mosquitoes were identified morphologically and molecularly. In 2021, Ae. j. japonicus was found in 14 out of 71 (19.7%) and Ae. koreicus in 58 out of 71 (81.7%) municipalities monitored and before negative. The mosquito was collected mainly in artificial containers located in small villages and in rural areas.

First detection of TBE in roe deer	In June 2021 the Belluno Province Police found a young female roe deer with neurological symptoms in the Belluno municipality, an area highly endemic for the presence of Tick borne encephalitis virus (TBEV). The animal was therefore humanely culled and promptly submitted to the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) for post-mortem examination and diagnosis. At necropsy, the macroscopic picture was inconclusive. RNA of TBEV extracted from roe deer brain was detected by real-time RT-PCR. Phylogenetic analysis revealed a close relationship to TBEV of the European subtype, and 100% similarity with a virus from the bordering Trento Province. The histological examination of the midbrain confirmed the viral etiology and specific immunofluorescence indicated the presence of a Flavivirus infection and characterized the pattern of infection in the neurons. This is the first case of clinical encephalitic manifestations due to TBEV in a roe deer.
Rapid identification of ticks species, genotyping and drug sensitivity testing of Borrelia spp. in North Estern Italy.	In 2021 within the framework of the research project funded by the Italian Ministry of Health RC IZSVE 08/20, more than 2000 Ixodex ricinus ticks (adults, nymphs and larvae) in 9 different localities of Belluno province (endemic area for Lyme borreliosis) were collected by dragging. In order to set up the isolation method for Borrelia strains, the ticks have been processed for cultivation using different broth and agar media suitable for the growth of Borrelia species. Were moreover set up and validated RT PCR methods for detection of Borrelia spp., Borrelia miyamotoi and Anaplasma phagocytophylum and those methods were applied on ticks homogenate used for the isolation procedure. Positive sample were subsequently genotyped by Multilocus sequence typing (MLST).
Genetic surveillance of SARS-CoV-2 in northeastern Italy (Part 1)	In order to provide insights into the evolutionary and epidemiological viral dynamics during the current COVID-19 pandemic in the Italian north-eastern region of Veneto, genetic surveillance of SARS-CoV-2 has been implemented since the beginning of 2020, with the complete genome sequences of about 5000 viruses available as of December 2021. All sequences obtained by Next Generation Sequencing on Illumina MiSeq and NextSeq platforms are promptly shared in the GISAID database (www.gisaid.org/) and in the Italian National Institute of Health platform ICoGen (https://irida.iss.it/). During the reporting period (January 2021 – December 2021), 125 different PANGO lineages and sublineages have been detected, including the five variants Alpha, Beta, Gamma, Delta and Omicron, recognized by WHO as Variant of Concern (VOC). Two marked shifts in the circulating lineages were observed in 2021: the first started in February 2021, with the replacement of B.1.177 and B.1.160 lineages with the Alpha variant, which soon became the prevalent one (85% prevalence in March 2021).
Genetic surveillance of SARS-CoV-2 in northeastern Italy (Part 2)	In April 2021 the Delta variant was detected for the first time in the region; as in the rest of the world, this variant spread rapidly in Veneto, reaching a frequency of 18% within a short period of time (May 2021), and being the only variant present in the region from August to November 2021. On 1st December 2021 the Omicron variant was detected for the first time in the region and since then a surge of cases caused by this high transmissible variant was observed in all provinces, indicating that a new variant replacement may be in progress. Differently, the VOCS Gamma and Beta have circulated only at low level throughout 2021. Specifically, the Gamma variant was identified for the first time in Veneto in January 2021, it persisted in the region until reaching a maximum prevalence of 8.5% in June 2021, and then disappeared definitively from August 2021. A similar trend was also observed for the Beta variant, whose prevalence in Veneto never exceeded 6.6%.

Harmonisation and integration of pan-Coronavirus surveillance in Italian wildlife (Part 1)	Within the framework of Strategic Current Research 1/20 "Susceptibility of mammals to SARS-COV-2: risks of reverse zoonosis and possibilities in translational medicine" with the IZSVe as lead partner, a surveillance plan has been implemented to study the circulation of coronavirus in Italian wildlife, including possible cases of reverse zoonosis by SARS-CoV-2, i.e. transmission from humans to animals. This activity involves the Institutes belonging to the IIZZSS network as well as Turin University, with the aim of ensuring a sampling effort over the entire national territory to obtain obtaining significant data for each species. Priority was given to the investigation of animal species i) belonging to animal families for which a higher susceptibility to SARS-CoV-2 has been assumed in the literature, ii) for which the circulation of coronaviruses was already known in the literature, ii) taxonomically related to domestic species associated with one or more coronavirus species.
Harmonisation and integration of pan-Coronavirus surveillance in Italian wildlife (Part 2)	We selected the following species: badger (Meles meles), marten (Martes foina) and, where available, other species belonging to the mustelid family, such as the red deer (Cervus elaphus), roe deer (Capreolus capreolus), fallow deer (Dama dama), chamois (Rupicapra rupicapra), wild boar (Sus scrofa), red fox (Vulpes vulpes), European hedgehog (Erinaceus europaeus), hare (Lepus europaeus) and all species belonging to the order of bats. In addition, hystrix (Hystrix cristata), marmot (Marmota marmot) and wolf (Canis lupus) were investigated in target areas. In order to investigate the tissue tropism of coronaviruses in the different species and to increase the sensitivity of the diagnostic protocol, it was decided to collect a gastrointestinal and a respiratory sample in duplicate for each animal.
Harmonisation and integration of pan-Coronavirus surveillance in Italian wildlife (Part 3)	For all bat samples and in the event of doubtful identification for mustelids, it was also recommended to carry out genetic identification of the host species from the organ extract. The IZSVe distributed to all partners two biomolecular methods, one for the identification of coronaviruses (and one for the genetic identification of the host species by amplification and sequencing of a portion of cytochrome oxidase I (COI). Up to now, among all partners of the project 1485 samples were analysed from 23 species, allowing for the preliminary identification of 47 positive samples. In particular, our laboratory identified 4 coronavirus belonging to the specie Erinaceous CoV in the hedgehog and one novel coronavirus in the badger, awaiting for further characterization.
Zoonoses	
Title of activity	**Scope**
BSE surveillance	Confirmation of epidemiological status of negligible BSE risk. We analysed 8434 cattle and none of them tested positive.
Brucellosis	Local application of the national surveillance programme to confirm the officially free status, by means of serology and abortion surveillance. We analysed 23574 ruminant sera, 400 aborted foetuses and 1395 bulk tank milk samples
SARS-CoV-2 surveillance on mink farms	Application of the O.M. dated December 13, 2021. We performed 21 necropies, we tested 92 sera, and 1722 realtime PCR
SARS-CoV-2 surveillance on dogs and cats	Application of the 0009224-17/04/2020-DGSAF-MDS-P (Italian Ministry of Health). We tested 199 cats and 154 dogs.

Q fever	Diagnostic services. We tested 4365 ruminant sera, and 523 samples (aborted foetuses, tank milk) with realtime PCR.
Current Research 'Mammalian susceptibility to SARS-COV-2: risks of reverse zoonosis and possibilities in translational medicine' (Part 1/5)	Following the COVID-19 emergency, the Ministry of Health allocated funds to carry out studies on mammals as a possible reservoir, incidental host and/or model of emerging coronaviruses, with particular focus on SARS-CoV-2 as the aetiological agent of the current pandemic. The project, of which IZSVe is leader partner, involves the collaboration of all the IIZZSS. The capillary distribution of these laboratories is a peculiarity of the National Health Service (SSN) that allows an accurate and widespread surveillance throughout the territory. In 2021 the IZSVe organized a kick-off meeting with the parties involved and formed working groups (WP) to work on the project's activities. These include serological surveillance in domestic animals, virological surveillance in wildlife, collection and distribution of tissues for immunohistochemical studies and immune studies on the hamster animal model. The IZSVe has also carried out several research activities whose preliminary results are here described.
Current Research 'Mammalian susceptibility to SARS-COV-2: risks of reverse zoonosis and possibilities in translational medicine'	One of the objectives of the project is to study the interaction between SARS-CoV-2 and the receptors of different domestic and wild animal species through in silico and in vitro investigations. The aim is to quantify and map ACE-2 receptors and possible co-receptors (e.g. TMPRSS-2) in the gastro enteric and respiratory tracts, combining confocal microscopy, immunohistochemical and biomolecular techniques.
WP1: Susceptibility to SARS-CoV-2. Evaluation of the distribution of ACE-2 receptors in animal tissues (Part 2/5)	The protein sequences of the ACE-2 receptor of the isoforms present in humans and in 9 species of domestic animals were compared, and in particular the conservation of 21 sites for SARS-CoV-2 binding was assessed. In the case of TMPRSS-2, 6 amino acids were taken into account, 3 of which make up the active site and 3 the substrate-binding site. In the case of ACE-2, the species showing the highest identity with human receptors were the cat and rabbit (85%), followed by the Syrian hamster (84%) and the ferret (83%). The analyses showed 82.6% identity between human and animal receptors, ranging from 81% to 85%.

Current Research 'Mammalian susceptibility to SARS-COV-2: risks of reverse zoonosis and possibilities in translational medicine' WP1: Susceptibility to SARS-CoV-2. Evaluation of the distribution of ACE-2 receptors in animal tissues (Part 3/5)	In the case of TMPRSS-2, the average identity was 78%, ranging from 73% to 87%, recorded between human and ferret and human and bovine, respectively. For most of the wild species of interest, however, analysis was not possible due to the absence of sequences in the available literature. Therefore, amplification and sequencing of the locus of interest will be carried out from tissue samples that will be analysed using a real-time method capable of quantifying receptor expression in lung and intestinal tissue. The mapping will then be confirmed by confocal microscopy. In this regard, an immunofluorescence method was developed from the tissues of Syrian hamster, cat and monkey.
Current Research 'Mammalian susceptibility to SARS-COV-2: risks of reverse zoonosis and possibilities in translational medicine'. WP2: Translational research. Sex-specific differences in SARS-CoV-2 immunopathogenesis using the Syrian hamster animal model (Part 4/5)	The Syrian hamster was used as a translational model to study variations in pathogenesis, immune response, incidence and severity of clinical manifestations and lesions, and to assess the duration and intensity of viral clearance in adult males/females. The use of the animal model was authorized by the Ethics Committee and Animal Welfare Board (OPBA) and by the Ministry of Health under authorisation code no. 1167/2020-PR. Briefly, 30 males and 30 eight-week-old females were used. 50% of the animals were used as negative controls, while the remaining individuals underwent experimental infection with SARS-CoV-2 administered via the intra nasal route using the alpha variant (i.e. English variant - lineage B.1.1.7). Each animal was examined and weighed daily for clinical signs of infection. Salivary swabs were taken daily and from each subject to verify SARS-CoV-2 elimination using biomolecular methods. At 2, 6 and 14 days post-infection (dpi) (key times according to the literature in the experimental infection of the golden hamster), 10 males and 10 females (5 from the infected group and 5 from the control group) were sacrificed for the collection of organs of interest such as the lung, intestine and CNS.

Current Research 'Mammalian susceptibility to SARS-COV-2: risks of reverse zoonosis and possibilities in translational medicine'.	Tissues were collected in RNA later (for gene expression analysis) and formalin (for immunofluorescence analysis and haematoxylin and eosin staining). A cardiac blood sample was also taken from these subjects in order to isolate by Ficoll gradient purification peripheral blood mononuclear cells (PBMCs), largely composed of monocytes and T, B and natural killer (NK) cells. On post-infection days (dpi) 2, 4, 6, 9 and 14, blood samples were taken from the gingival vein to study cytokine release and the development of neutralising antibodies. All animals present at the time of sampling were sampled, i.e. 30 males and 30 females at 2 dpi, 20 males and 20 females at 4 and 6 dpi and 10 males and 10 females at 9 and 14 dpi.
WP2: Translational research. Sex-specific differences in SARS-CoV-2 immunopathogenesis using the Syrian hamster animal model (Part 5/5)	Biological samples will be used to compare the immune response of males and females trough transcriptomic analyses and identifying the dynamics of cytokines and neutralizing antibodies in the serum. Analyses are ongoing.

ConVergence Project: Assessing swine as potential hosts for emerging Coronaviruses (Part 1)	ConVergence Project: Assessing swine as potential hosts for emerging Coronaviruses The EU and the Italian Ministry of Health, in collaboration with donors from the Netherlands (Erasmus Medical Centre – Viroscience) and the United Kingdom (University of Sussex) have financed the ConVErgence project within the framework of the ICRAD call to investigate the process of emergence of coronaviruses in the swine industry, focusing on bats and humans as the most likely sources of infection. Currently, there are seven CoV species known to infect humans, among which three are endemic and cause the seasonal flu and one is responsible of the current pandemic. On the other hand, coronaviruses are extremely diversified and frequent in bats, to the point that most mammalian coronaviruses seem to derive from the pool of bat CoVs, exception made for a large cluster of viruses that includes several species common in humans and in livestock, including pigs, but have never been described in bats.

ConVergence Project: Assessing swine as potential hosts for emerging Coronaviruses (Part 2)	It can be assumed that swine in closer contact with bat and humans are more likely to be exposed to their viruses, and that recurrent exposures increase the possibility of an effective cross-species transmission. The objective of ConVErgence is i) to investigate the relationship between swine and either bats or humans in different farming systems, ii) to determine to what extent pigs are exposed to known bat and human CoVs by measuring their antibody response and iii) to trace the circulation of bat/human CoVs in pig populations from North Eastern Italy. In addition, we will consider the fact that shedding of coronaviruses is highly seasonal in both bats and humans, and field data from bats and swine will be used to build a mathematical model of the spillover to identify the situations more at risk. Should the study succeed in the identification of novel CoVs, of new variants or of viruses associated with novel hosts, we will characterize their genome, their adaptation and their ability to cause harm to the new host and investigate their potential of infecting other species.ConVErgence will use cutting-edge technologies from different fields including veterinary medicine, ecology, virology, epidemiology and mathematical modelling by drawing from the expertise of the partners included in the consortium with the Istituto Zooprofilattico Sperimentale delle Venezie, namely the University of Sussex and the Erasmus Medical Centre in Rotterdam.
Different immune response in adult and paediatric patients in response to natural SARS-CoV-2 infection	In collaboration with the Division of Pediatric Infectious Diseases, Department for Women's and Children's Health of the University of Padua (Italy) a single-center, prospective observational study was conducted on 57 family clusters of coronavirus disease 2019, including children of neonatal and pediatric age attending the University Hospital of Padua (Italy). For each patient, blood samples were collected for both the quantification of neutralizing antibodies (nAbs) through a plaque reduction neutralizing test and the detection of antinucleocapsid-spike protein immunoglobulin G and/or immunoglobulin M. Analysed sera of 152 confirmed COVID-19 cases (82 parents and 70 children or older siblings of median age of 8 years, interquartile range: 4–13), presenting asymptomatic or mildly symptomatic disease, showed that despite the decrease of immunoglobulin G over time, nAbs were found to persist up to 7 to 8 months in children, whereas adults recorded a modest declining trend. Interestingly, children aged <6 years, and, in particular, those aged <3 years, developed higher long-lasting levels of nAbs compared with older siblings and/or adults. The results of the collaborative work, which added knowledge on nAbs kitetics in children, was object of scientific publication on the specialized Journal Pediatrics.
Wildlife	
Title of activity	**Scope**
Surveillance of trichinellosis in wild reservoir hosts	13375 muscle samples from wild boars (11982), red foxes (868), mustelids (479), golden jackal (32) and wolves (14) have been examined during 2021, with negative results.

Use of the Webgis named “Visore” to support the epidemiology and surveillance of trichinellosis	More than 10000 foxes and other wild carnivores, from 2005 to 2020 are uploaded to WebGIS named “Visore” to support the surveillance of trichinellosis. In this period, Trichinella britovi was found in 10 red foxes. Preliminary spatial analysis was performed to identify possible Trichinella clusters, thus defining a surveillance strategy and introducing a risk-based component.
in North-East Italy	Not representative clusters to target a more intensive surveillance aimed at identifying risk factors for the presence and maintenance of this parasite has been found. In the future, we plan to use the WebGIS to plan active surveillance on a local scale where the parasite had been identified in the past, to increase the likelihood of its detection and estimate a maximum possible prevalence.

Development of a WebGis for surveillance of pan-Coronavirus in wildlife	In 2021, a WebGIS was developed to georeference the samples and visualize data related to the Pan-Coronavirus in wildlife. Data acquisition was made through a dedicated editing tool available in the dynamic map. The WebGIS was developed using the JS OpenLayers3 framework, for the front-end part, and in Java for the back-end side. The software is published through the Tomcat 7 application server, on a Linux server.
Characterisation of a West Nile Virus isolated in Province of Padua (Part 1)	West Nile virus (WNV) is a vector-borne Flavivirus that infects mainly mosquitoes, birds, horses and humans. WNV is responsible for asymptomatic infections, but can also lead to a variety of clinical manifestations ranging from mild fever to neuroinvasive disease. The frequency and severity of WNV-related diseases have recently increased in the European Union and neighbouring countries, with particular concern for the Mediterranean area. WNV is endemic in northeastern Italy and is considered particularly dangerous for certain risk categories (i.e immunocompromised patients, the elderly, children). In 2021, six pools of Culex pipiens from the provinces of Padua (samples 1-4), Verona (sample 5) and Treviso (sample 6), positive for WNV lineage I (samples 1-4) and WNV lineage II (samples 5-6) were genetically characterised. All samples were processed with two protocols for amplification of WNV complete genomes by target PCR specific for the two lineages; the resulting amplicons were sequenced with Next Generation Sequencing (NGS) approach.

Characterisation of a West Nile Virus isolated in Province of Padua (Part 2)	Using the protocol specific to lineage I, the complete sequence of samples 1-4 was obtained. By performing phylogenetic analysis, we observed that these viruses showed the highest similarity (98.4%-98.5%) with a virus isolated in France in 2015 (MT863559). When compared to the other lineage I viruses isolated in Italy between 2008 and 2013, however, samples 1-4 showed a similarity of 97.5%-98.3%. Using the protocol specific to lineage II, we obtained the complete sequence of samples 5-6 and a partial sequence for samples 1-4 (41.19% of the genome), indicating the presence of a co-infection of WNV belonging to the two different lineages in these samples. Phylogenetic analysis of these viruses showed that they cluster with viruses identified in Italy between 2016 and 2019.
The role of stranded marine mammals in the human-animal interface (part 1)	Cetacean stranding data can provide baseline information on marine ecosystems and factors affecting marine animal health. Futhermore marine mammals can be infected with or be healthy carriers of pathogens which are transmissible to humans and/or come from humans. Among the best known pathogens we find Poxvirus, influenza virus, Calicivirus, West Nile virus, and, potentially, Coronavirus, Papillomavirus, Herpesvirus, Norovirus and Morbillivirus. Marine mammals are also susceptible to pathogenic bacteria, such as Brucella spp., some species of mycoplasms and to parasites (in particular Toxoplasma gondii). The Italian NRC for Diagnostic Activities in Stranded Marine Mammals (C.Re.Di.Ma.) coordinates surveillance and control activities performed by National Public Health Services (Istituti Zooprofilattici network) and other RC at the AHI.
The role of stranded marine mammals in the human-animal interface (part 2)	In 2021 the IZSVe has been involved in a new research project funded by the Italian Ministry of Health, for the detection of zoonotic diseases in samples of stranded marine mammals. Analyses of samples collected from 9 stranded cetaceans were performed for detection of: Influenza A virus (Nagy et al. 2020;Heine et al 2015), West Nile virus (C.E.S.M.E Protocol), Morbillivirus (Verla et al. 2017), Betanodavirus(Baud et al 2015), Coronavirus (Drzewniokova et al 2021), Astrovirus (Chun et al 2008), Herpesvirus (VanDevanter et al 1996) and SARS-CoV 2 (Corman et al 2020). In well preserved samples further analyses were performed: isolation in MDK-Slam dog cells for detection of CeMV ; metagenomic analysis using next-generation-sequencing (NGS) and bioinformatic analysis for the detection of viral RNA and DNA. Where required bacteriological analyses were also carried out. All samples tested negative.
Diagnosis, biotechnology and laboratory	
Title of activity	**Scope**
Detection of Trichinella spp. in domestic animals	72225 swine and 4650 equine regularly slaughetered have been controlled for the presence of trichinella larvae in muscle samples

Standardization and validation of molecular diagnostic tests for E.multilocularis	During 2020-21, in collaboration with UNIPI, FEM and UNIPD, 123 fox carcasses were examined for Echinococcus multilocularis infection to infer sensitivity and specificity of the diagnostic approach implemented for parasitic surveillance (sedimentation/filtration followed by multiplex PCR on positive samples) and a novel real-time PCR (whole stool qPCR) protocol, using intestinal scraping technique as a gold standard. As qPCR test resulted far more efficient in Echinococcus multilocularis detection in fox feces, with a sensitivity of 83% versus 21 % of PCR, it was adopted by IZSVe for current and future routinary surveillance. All adult Echinococcus multilocularis specimen found during the validation were collected and will be analyzed for a genetic characterization of the parasitic strain. Also, other cestode species found were collected and will be morphologically analyzed. For Bolzano province, true prevalence in foxes was also estimated using a sample of 235 environmental feces, allowing to reconsider the provincial territory as a highly infected area at approximately 14% prevalence.
Diagnosis of zoonosis transmitted by ticks, sand flies, fleas	2001 samples of arthropod vector specimens have been tested for transmitted pathogens

Hantavirus diagnostic activities (Part 1)	Hantaviruses are enveloped RNA viruses of the Bunyaviridae family, whose genome consists of single strands of negative-strand RNA. The genus Hantavirus includes 23 species classified by the International Commission on Taxonomy of Viruses (ICTV), and about 30 other viruses for which a complete characterisation is not yet available. Hantaviruses are of public health interest because they are pathogenic to humans, where mortality can be as high as 40%, depending on the virus species. Most hantaviruses are maintained in nature by rodents (order Rodentia), although the detection of viral species in bats (order Chiroptera) or insectivores (order Soricomorpha) has dramatically increased. Nevertheless, each species of hantavirus seems to be specifically associated with its reservoir host; in Europe the yellow-necked field mouse (Apodemus flavicollis) and the reddish vole (Myodes glareolus) are respectively the reservoirs of Dobrava-Belgrade (DOBV) and Puumala (PUUV) viruses.
Hantavirus diagnostic activities (Part 2)	Human infection occurs by inhaling virus particles from the saliva, or through urine and/or faeces. Several scientific works have underlined a relevant correlation between population dynamics in the host species and the occurrence of human cases. In particular, it is reported that pullulation (a massive increase in population density) can generate a hantavirus epidemic peak, resulting in increased human exposure. The fact that pullulation is linked to environmental factors such as higher temperatures and seasonal changes in rainfall patterns highlights that human hantavirus diseases must be treated through an integrated approach between doctors, veterinarians and ecologists, in full respect of the One Health concept.
Other (Name the category)	
Title of activity	**Scope**
Developing and validating serological diagnostic methods to identify SARS-CoV-2 antibodies in susceptible animals (Part 1)	The Plaque Reduction Neutralisation Assay (PRNT) was validated using experimental (rabbit and hamster) and field (fur mink herd positive) sera, the gold standard to define the immunological profile of infected animals and quantify the presence of neutralising antibodies. Over 100 sera from companion animals (dogs, cats, pet minks) and ˃ 200 sera from farm animals (cattle, rabbit, sheep, fur mink) were tested within the framework of collaborative studies with universities/national/international groups and thanks to current research projects financed by the Italian Ministry of Health and having the IZSVe as project leader. The PRNT method visualises the viral growth on 96-well plates over a period of about 23 hours by immunocytochemical staining, using a monoclonal antibody specific to the nucleocapsid protein (NP) of the SARS-CoV-2 virus. The microtiter allows a high scaling of the method to use a reduced and serially diluted serum volume (< 50 microlitres) to obtain an antibody titre, making this an extremely useful method to analyse a great number of samples and assess their different neutralising capacity.
Developing and validating serological diagnostic methods to identify SARS-CoV-2 antibodies in susceptible animals (Part 2)	The samples were then used to validate protocols based on the fluid phase luciferase immunoprecipitation systems (LIPS), a technique developed by the Joint FAO/International Atomic Energy Agency Center (IAEA – Vienna, Austria) to detect antibodies against the N or S proteins of the SARS-CoV-2 virus. LIPS offers numerous advantages, as it requires a very small volume of serum (about 1 microlitre) and is characterised by a rapid analysis, reduced to a few hours. Our tests revealed 100% concordance between the LIPS-S test and the PRNTs and only a modest correlation between LIPS-N and PRNTs. Results were published in Viruses in 2021.

Developing and validating methods to assess antiviral compounds against SARS-CoV-2	As part of the response to the COVID emergency, several in vitro methods were developed to preliminary evaluate compounds with antiviral activity against SARS-CoV-2. Such methods were then adapted to evaluate compounds with specific action against SARS-CoV-2 in order to produce a rapid, yet highly accurate screening method to define the efficacy of antiviral compounds. The screening panel included: (i) the assessment of the cytotoxicity of the compound in different animal (VeroE6) or human (CaCo-2 or CaLu-3) derived cell lines; (ii) the plaque size reduction assay (PRA) to evaluate a broad spectrum of compound concentrations and to define an efficacy range (EC50); (iii) the virus yeld reduction assay (VYRA) for an accurate assessment of the ability of a compound to inhibit viral growth at a given concentration. Analysis of results allowed to calculate a selectivity index (SI) which describes the distance between effective and toxic concentrations.
Development and validation of an animal model for the evaluation of antiviral compounds against SARS-CoV-2 (Part 1)	In response to the ongoing COVID emergency, researchers at the IZSVe validated an in vivo animal model to evaluate the efficacy of the most promising antiviral compounds against SARS-CoV-2. The selected animal model was the Syrian hamster (Mesocricetus auratus), identified based on the extensive literature available in accordance with the 3R principles of using the animal model with the lowest neurological development. The Syrian hamsters' immune response to infectious pathogens is similar to humans and, as such, they are chosen to study the replication mechanisms of several zoonotic viruses, including West Nile virus (WNV), viruses belonging to the alphavirus genus (Eastern, Western and Venezuelan equine encephalitis viruses) or even Hendra and Nipah. This animal model has proved extremely useful also to investigate therapeutic approaches such as passive immunisation or compounds with an antiviral action, aimed at reducing the severity of the disease itself.
Development and validation of an animal model for the evaluation of antiviral compounds against SARS-CoV-2 (Part 2)	Among the species susceptible to SARS-CoV-2 infection, the hamster model proved to be highly susceptible to infections caused by circulating strains without the need of prior adaptation. Four promising antiviral compounds were assessed and identified by in vitro testing, with the aim of identifying an effective therapy for SAR-CoV-2 disease in humans. Thankfully, we were able to gather crucial information on the ability of the tested compounds to reduce (i) the viral titre in the affected tissues, (ii) the degree of inflammation, (iii) the severity of any lesion and (iv) the extent of clinical symptoms following infection. Relevant information about immune response to SARS-CoV-2 infection were collected during antiviral trials and will be instrumental for future research on vaccine development.

Development and validation of a method to study the ability of viral internalisation and undressing of the host cell	Within the framework of the ZIKAction project funded by the Horizon 2020 programme, we were able to further study the pathogenetic mechanism of Zika virus replication in human placenta. Data from previous trials showed that Zika strains grown in mammalian cells (ZIKV-V), when compared to the same strains originating from insect cells (ZIKV-C6), have a higher infectious capacity in the placenta explants. The phenotypic difference between the two viruses was attributed to the different lipid composition of the outer envelope of the virus particles. To assess how the structural difference in viral lipids could influence Zika infectivity in human placental trophoblasts we tested these two ZIKVs strains for their capacity to bind and enter into immortalized placenta cells. We labelled viral membrane of ZIKV-V and ZIKV-C6, using a lipid marker (R18) able to release a signal at the moment of viral entering into the cell host cytosol. Our results showed that the two viruses had the same host receptor binding capacity but ZIKV-V reported a significant delay in entering into the cell cytosol compared to ZIKV-V. This evidence demonstrated that the ZIKV replicative efficiency in placenta trophoblasts is strictly related to its capacity of entering into cell cytosol and depends on the lipid composition of cell and viral membranes.
Developing a translational mouse model to assess the impact of dietary lipids on the metabolism and placental structure in pregnant women	Data collected so far on the vertical transmission of the Zika virus in pregnant women have highlighted the important role of lipids in viral pathogenesis at placental level. In consideration of this, we were wondering whether the quality and quantity of lipids consumed in the diet during gestation could affect lipid composition of women placenta a consequently their susceptibility to Zika infection. To answer this question, we are validating a mouse model capable of mimicking the gestation of healthy women with different diets in terms of lipid content. Female C57BL/6J mice were fed with high or low fat diets for a month and then were mated and sacrificed at 15 gestation-days to collect, serum, liver and placental tissue. Data on sera and liver highlighted an increase of cholesterol and HDL in mice fed with high fat diets, while lipidomic analysis on placental tissues are ongoing. Interestingly, high fat diet lead to a significant increase of miscarriages percentage compared to diets with a low-fat content.
Development and validation of a PCR pan-Coronavirus	In order to select the best protocol for broad-spectrum CoV surveillance in animals, IZSVe compared the performance of primers sets from the literature both in silico and, for four mostly used methods, also in vitro, using four isolates of α, β and γ-CoV. In silico, only primers developed for bird surveillance by Chu et al. (2011) showed sufficient complementarity with δ and γCoVs, as confirmed in vitro using IBV. However, the Chu's (2011) protocol was not optimal for α- and β-CoVs, including viruses of major interest for animal and public health. All nested protocols showed poor performance in the first step, with best results obtained with primers from Hu et al. (2018), developed as a one-step approach. We combined primers from Hu (first step) and Chu (nested), trying to balance sensitivity and broad-spectrum. The new protocol showed the best sensitivity for α- and β-CoVs and improved performance for IBV. In order to test the field performances of this protocol, we analysed archive positive samples and we were able to confirm the infection from all of them, including oral swabs positive for SARS-CoV-2 at Ct values up to 30.76 and feces positive for avian deltacoronaviruses. All samples were sequenced using a Sanger approach, and yielded clean sequences of approximately 440 base pairs that were successfully used to characterise the detected virus from a genetic perspective.

Implementation and preliminary validation of a Pan-Hantavirus PCR (Part 1)	The IZSVe acquired a biomolecular method capable of identifying all species of the genus Hantavirus, in order to respond to the diagnostic suspicion due to an increased mortality among wild rodents in the territory. The nested RT-PCR protocol developed by Klempa and colleagues in 2006 was selected and used as reference method directed towards the L segment encoding for RdRp polymerase. Complementarity between primers and viral sequences was tested in silico using an alignment of 15 reference sequences. The analysis was implemented on Geneious Prime by setting a limit of 4 mismatches, none of which located within 3 bp of the 3' end of the primer. The analysis revealed a few mismatches and was considered ideal to identify the Puumala and Dobrava-Belgrade species, which are of greatest interest as they are zoonotic and widespread in wild rodents in Europe. In order to standardise the method, two different dilutions of primers were tested for the first amplification cycle. Their performance was compared using two positive reference samples (Dobrava-Belgrade and Puumala species) provided by the Department of Molecular Medicine of the University of Padua.

Implementation and preliminary validation of a Pan-Hantavirus PCR (Part 2)	The protocols used showed comparable sensitivity on the reference samples, amplifying 2/2 repeats for all dilutions for the Dobrava-Belgrade strain and for the first 4 dilutions of the Puumala strain, amplifying 1 in 2 replicates in case of a fifth dilution. This study showed that the use of a reduced concentration of primers during the first amplification cycle significantly reduced background noise, resulting in clearer nested PCR bands and better sequencing performance, which is necessary for virus typing. The selected protocol was further tested in vitro to define analytical sensitivity, using serial dilutions of synthetic RNA from Puumala and Andes viruses, prepared in molecular biology water based on RNA quantifications performed in Nanodrop and Qubit. Each dilution was prepared and tested in triplicate and the LOD was identified as the concentration at which PCR was positive in all repeats. This preliminary validation shows that the method is capable of identifying up to a minimum of 10 RNA copies per microlitre of both viruses using the full nested protocol.

ToR : To propose or develop methods and procedures that facilitate harmonisation of international standards and guidelines applicable to the designated specialty

2. Proposal or development of any procedure that will facilitate harmonisation of international regulations applicable to the surveillance and control of animal diseases, food safety or animal welfare

Proposal title	Scope/Content	Applicable area
xx	xx	☐Surveillance and control of animal diseases ☐Food safety ☐Animal welfare

ToR: To establish and maintain a network with other OIE Collaborating Centres designated for the same specialty, and should the need arise, with Collaborating

Centres in other disciplines

ToR: To carry out and/or coordinate scientific and technical studies in collaboration with other centres, laboratories or organisations

3. Did your Collaborating Centre maintain a network with other OIE Collaborating Centres (CC), Reference Laboratories (RL), or organisations designated for the same specialty, to coordinate scientific and technical studies?

Yes

Name of OIE CC/RL/other organisation(s)	Location	Region of networking Centre	Purpose
National Reference Centre for Leptospirosis	Brescia, Italy	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Research project, Epidemiological data sharing, submission of Leptospira strains/DNAs for genotyping, proficiency testing
National Reference Centre for Antrax (Centro di Referenza Nazionale per l'Antrace (Ce.R.N.A.)	Foggia, Italy	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Diagnosis confirmation, training, proficiency testing
OIE Reference Laboratory for Brucellosis, National Reference Centre for Brucellosis	Teramo, Italy	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Diagnosis confirmation, proficiency testing
National Reference Centre for Leishmaniosis (C.RE.NA.L.)	Palermo, Italy	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Proficiency testing
OIE Reference Laboratory for Swine Influenza	Parma, Italy	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Research project
National Reference Centre for Foreign Diseases of Animals	Teramo, Italy	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Diagnosis confirmation

Aedes Invasive Mosquitoes Cost (AIM-COST)	Europe	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Research project
National Centre for Foreign Animal Disease of the Canadian Food Inspection Agency (NCFAD)	Winnipeg (Canada)	☐Africa ☒Americas ☐Asia and Pacific ☐Europe ☐Middle East	Research studies focusing, among the others, on investigating zoonotic viral agents including animal influenza viruses

4. Did your Collaborating Centre maintain a network with other OIE Collaborating Centres, Reference laboratories, or organisations in other disciplines, to coordinate scientific and technical studies?

Yes

Name of OIE CC/RL/other organisation(s)	Location	Region of networking Centre	Purpose
Medical Research Council University of Glasgow Centre for Virus Research - CVR (OIE CC Viral Genomics and Bioinformatics)	Glasgow (United Kingdom	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	OIE-ad hoc group on high throughput sequencing, Bioinformatics and computational Genomics (HTS-BCG)

Australian Animal Health Laboratory . CSIRO Livestock Industries (OIE CC Laboratory Capacity Building)	Victoria (Australia)	☐Africa ☐Americas ☒Asia and Pacific ☐Europe ☐Middle East	OIE-ad hoc group on high throughput sequencing, Bioinformatics and computational Genomics (HTS-BCG)
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini"- IZSLER (OIE CC Veterinary Biologicals Biobank)	Brescia (Italy)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	OIE-ad hoc group on high throughput sequencing, Bioinformatics and computational Genomics (HTS-BCG)
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri"	Roma (Italy)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Surveillance activities for West Caucasian Bat Lyssavirus (WCBV) in the Tuscany region

Irish Equine Centre (IEC) (OIE RL for equine influenza)	Kildare (Ireland)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	FAO-OIE Advisory Group on viral evolution of SARS-CoV-2 in animals
Royal Veterinary College (RVC) (OIE CC for Risk Analysis & Modelling)	London (UK)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	FAO-OIE Advisory Group on viral evolution of SARS-CoV-2 in animals
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (National Reference Centre for Whole Genome Sequencing of microbial pathogens: database and bioinformatic analysis)	Teramo (Italy)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Sequencing of the SARS-COV-2 genome in humans

Ospedale Sacro Cuore Don Calabria (IRCCS - Scientific Institute for Research, Hospitalization and Healthcare)	Verona (Italy)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	SARS-COV-2 sequencing data and analysis support; Intra-host variation and evolutionary dynamics of SARS-CoV-2 in patients; Comparison of saliva and nasopharyngeal swab testing methods
University of Liverpool	Liverpool (UK)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Research collaboration for diagnostic and scientific purposes on animal and human viral agents

Val d'Hebron University Hospital (Spain) ZIKAction, Horizon2020 - Grant Agreement 734857 of the EU Commission DG for research and innovation	Coordinator: INSERM (France)	☐Africa ☐Americas ☐Asia and Pacific ☒Europe ☐Middle East	Interdisciplinary programme of research studies to address key knowledge gaps related to ZIKA epidemiology, natural history and pathogenesis, focusing on maternal and child health

ToR: To place expert consultants at the disposal of the OIE.

5. Did your Collaborating Centre place expert consultants at the disposal of the OIE?

Yes

Name of expert	Kind of consultancy	Subject
Monne Isabella	Meetings of the FAO-OIE Advisory Group on SARS CoV-2 evolution in animals (January and March 2021)	Purpose of the group is to advise on risks related to the evolution of SARS-CoV-2 in animal populations, in addition to liaising with the WHO viral evolution group. Specifically: to monitor and assess the latest information on SARS-CoV-2 viral evolution in animals; keep an inventory of observed mutations and assess their implications for animal and/or public health; list knowledge gaps and priority research areas in relation to SARS-CoV-2 viral evolution

Monne Isabella	5th and 6th call of the FAO-OIE Advisory Group on SARS CoV-2 evolution in animals (September and December 2021)	(See above)

ToR: To provide, within the designated specialty, scientific and technical training to personnel from OIE Member Countries

6. Did your Collaborating Centre provide scientific and technical training, within the remit of the mandate given by the OIE, to personnel from OIE Member Countries?

Yes
a) Technical visits: 0
b) Seminars: 0
c) Hands-on training courses: 0
d) Internships (>1 month): 1

Type of technical training provided (a, b, c or d)	Content	Country of origin of the expert(s) provided with training	No. participants from the corresponding country
d	Thesis on coronaviruses affecting Italian wildlife (within a research funded by the Italian Ministry of Health)	Italy	1

ToR: To organise and participate in scientific meetings and other activities on behalf of the OIE

7. Did your Collaborating Centre organise or participate in the organisation of scientific meetings on behalf of the OIE?

No

ToR: To collect, process, analyse, publish and disseminate data and information relevant to the designated specialty

8. Publication and dissemination of any information within the remit of the mandate given by the OIE

that may be useful to Member Countries of the OIE

a) Articles published in peer-reviewed journals: 34

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