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MEETING OF THE OIE *AD HOC* GROUP ON EQUINE TRYPANOSOMOSES[1]

Paris, 14-16 June 2016

A meeting of the OIE *ad hoc* Group on (non-tsetse transmitted) equine trypanosomoses (hereafter the Group) was held at the OIE Headquarters from 14 to 16 June 2016.

1. Opening

On behalf of Dr Monique Eloit, Director General of the OIE, Dr Brian Evans, Deputy Director General and Head of the Scientific and Technical Department, welcomed and thanked the Group for its efforts in reviewing the *Terrestrial Animal Health Code* (hereafter the *Code*) chapter on dourine and draft *Code* chapter on surra.

Dr Evans reminded the Group that dourine and surra were both OIE listed diseases, however recommendations for trade in live susceptible animals and their products, were currently only provided in the *Code* for dourine. He informed the Group that OIE Member Countries had expressed their need for trade standards applicable to surra as well, especially in the context of the OIE initiative with the International Equestrian Federation (FEI) and the International Federation of Horseracing Authorities (IFHA) for the facilitation of international movement of competition horses.

Dr Evans informed the Group that a previous OIE *ad hoc* Group on equine trypanosomoses had been conveyed in 2015 to draft a *Code* Chapter on surra and revise the *Code* Chapter on dourine. The report of this *ad hoc* Group was not endorsed by the *Scientific Commission for Animal Diseases* (hereafter the Scientific Commission) nor discussed by the *Terrestrial Animal Health Standards Commission* (hereafter the Code Commission); a new *ad hoc* Group has therefore been conveyed to finalise this task.

Dr Evans emphasized that the proposed standards should be pragmatic, based on risk mitigating approaches and on the best available science. Lastly, Dr Evans insisted on the importance of a detailed meeting report highlighting the scientific justifications of the proposed texts, as meeting reports are the main channel to communicate the rationale of the proposed standards to the Scientific and Code Commissions and to OIE Member Countries.

2. Adoption of the agenda and appointment of chairperson and rapporteur

In the absence of a member of the Group volunteering to chair the Group, Dr Baptiste Dungu, representative of the Scientific Commission, was exceptionally appointed as a Chair. Dr Charles E. Lewis acted as rapporteur. The Group adopted the proposed agenda.

The agenda and list of participants are presented as Appendices I and II, respectively.

[1] Note: This *ad hoc* Group report reflects the views of its members and may not necessarily reflect the views of the OIE. This report should be read in conjunction with the September 2016 report of the Scientific Commission for Animal Diseases because this report provides its considerations and comments. It is available at: http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/scientific-commission-reports/meetings-reports/

3. Presentation of the comments of members of the Scientific Commission and Code Commission on the report of the previous *ad hoc* Group

Dr Dungu clarified that the work of the *ad hoc* Group on equine trypanosomoses that met in 2015 could be utilized by the Group as the reference document for discussion. He emphasised the need to further elaborate on it to fully meet Member Countries' expectations to resolve trade issues associated with equine trypanosomoses.

Dr Etienne Bonbon, President of the Code Commission, advised the Group to specifically concentrate on providing practical and science based guidance to Member Countries to manage surra and dourine, especially in the context of international trade.

4. Revision of the scope of the *Code* chapters

The Group extensively discussed the infections caused by trypanosomes in equids.

The Group reviewed the following article: Carnes J. *et al.* (2015) "Genome and phylogenetic analyses of Trypanosoma evansi revealed extensive similarity to *T. brucei* and multiple independent origins for dyskinetoplasty." *PLoS Negl Trop Dis.,* **9**(1): e3404 that describes that three out of the four known groups within the Trypanozoon subgenus cause the disease dourine. Unpublished data support that the Italian dourine outbreak was actually caused by a trypanosome very similar to *T. brucei* and *T. evansi* type B rather than *T. equiperdum*.

The group also reviewed the following articles: Claes Buscher *et al.* (2005) "*Trypanosoma equiperdum*: master of disguise or historical mistake?" *Trends in Parasitology*, **21**(7): 316-321 (a review with the proposal of a new definition for Dourine) and Zablotskij V.T., *et al*. (2003) "The current challenges of dourine: difficulties in differentiating *Trypanosoma equiperdum* within the subgenus Trypanozoon." *Rev. sci. tech. Off. int. epiz.*, **22**(3), 1087-1096.

Data from an unpublished project conducted by the United States Department of Agriculture (USDA) was also shared with the Group and discussed (unpublished report on the comparison of three reference isolates of *Trypanosoma equiperdum* in ponies).

The Group concluded that these studies converged in indicating that: (i) there is little genetic distinction between *T. evansi, T. equiperdum,* and *T. brucei,* (ii) clinical distinction of individual cases into surra or dourine is not possible, (iii) differential laboratory diagnostics of the infections are complex.

The Group therefore recommended combining the infection of equids with parasites of the subgenus Trypanozoon (*T. evansi, T. equiperdum, or T. brucei*) into a specific *Code* chapter. For consistency, the Group also noted that equids should be excluded from the draft *Code* chapter on infection with *T. evansi* (draft chapter 8.X). Under those provisions, Member Countries would report any infection with trypanosome in equids as an "infection with Trypanozoon in equids".

In brief, the Group determined that the best course of action was:

- To revise the current *Code* Chapter 12.3. on dourine to encompass all infections with Trypanozoon in equids;
- To dedicate the draft *Code* Chapter 8.X. to the infection of susceptible species other than horses with *T. evansi* (non-equine surra).

In drafting Chapter 8.X and revising Chapter 12.3, the Group routinely referred to the report from the *Meeting of the OIE Ad hoc Group on Equine Trypanosomoses – Paris, 21-23 July2015*.

5. Draft Chapter 8.X. (Infection with *Trypanosoma evansi* – non equine surra)

Discussions on individual articles were as follows:

– In **Article 8.X.1 (General Provisions),** the *Code* chapter drafted in 2015 mentioned that "*few human cases have been described*". The Group clarified that the rare occurrence of cases of human infection with *T. evansi* was associated with the lack of serum factors that would normally have destroyed the parasite in the serum (ApoL1 lytic factor). The Group agreed that the General Provisions should concentrate on the

facts and evidence supporting the recommendations to mitigate the risk of spread of infection in animals, including thorough management of outbreaks and safe trade in live susceptible animals and their products. Therefore, while acknowledging that the possible occurrence of cases of human infection was relevant in a public health perspective, the Group decided not to mention it in Article 8.X.1, since measures to prevent human cases of that infection were out of the scope of the chapter.

The Group debated the incubation period for infection with *T. evansi.* Due to the wide range of susceptible hosts, the incubation period is highly variable. The Group eventually determined that the best course of action was to utilise the maximum timeframe of six months.

The *Code* chapter drafted in 2015 stated that *T. evansi* can survive for one to two days in stomoxes and 72 hours in infected meat. Based on scientific evidence[2], the Group recommended revising the duration of survival of the parasite in stomoxes to 72 hours. Regarding infected meat, the Group was unable to find specific references in regard to the survival of the parasite for up to 72 hours. However, it was decided to leave this statement as the Group could not justify its removal without further clarification. In addition, considering that carnivores can be contaminated through the contact of the oral mucosa with the parasite contained in ingested fresh meat from infected animals (cases of stray dogs scavenging on slaughterhouse waste), the Group recommended that standard processing practices should be complied with in order to mitigate the risk of transmission through this route -including the prevention of contact between animal by-products and carnivores-.

- The Group established the list of safe commodities in **Article 8.X.2 (Safe Commodities)** on the basis of current knowledge[3].

- **Article 8.X.3 (Country or *zone* free from infection with *T. evansi* in one or more animal species)** was reviewed and the Group decided to include the possibility for a country to claim freedom in specific animal species.

  Regarding the conditions for freedom recognition, the Group discussed referencing the point a of Article 1.4.6.1 that specifically addresses historical freedom (i.e. last occurrence of the infection more than 25 years ago) or the whole Article 1.4.6.1 (i.e. including point b that provides requirements to be complied with for at least 10 years to declare a country or a zone free from disease or infection if cases have occurred within the last 25 years). The Group decided that reference should be made to the whole Article 1.4.6.1 since the provisions for historical freedom alone would not suffice.

  Point 2 of Article 8.X.3 requires that a free country or zone, adjacent to an infected one, should conduct adequate surveillance in an area of appropriate distance from the bordering infected country or zone in order to detect any case of infection with *T. evansi.* The Group discussed what should constitute an "appropriate distance" and agreed that it should be deemed appropriate in regard to the specific location of the concerned countries or zones, taking into consideration numerous factors such as the vector ecology, the epidemiological situation, the geographic isolation, etc. The Group therefore recommended that this distance should be defined by the Member Country based on an assessment of the relevant local parameters.

- **Article 8.X.4 (Recovery of free status)** was extensively discussed and reformatted. This article gives the possibility to handle an outbreak situation either by applying a stamping out policy or by treating infected or serologically positive animals. The Group insisted that alternatively, if these conditions could not be complied with, the recovery of the free status may also be based on the conditions provided in Article 8.X.3.

  The Group discussed the feasibility of a stamping out policy in the light of the definition approved by the Word Assembly during the 84th General Session in May 2016. This definition includes *"the cleansing and disinfection of establishments"*, however, it was unclear to the Group if the definition also includes disinsection/disinfestation as part of a stamping out policy. If the newly adopted definition does not account for this, the Group recommended that it should be incorporated.

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2 Baldacchino F. *et al. (2013).*- Transmission of pathogens by Stomoxys flies (Diptera, Muscidae): a review. *Parasite*, **20**: 26.

3 Desquesnes M. *et al*. (2013).- *Trypanosoma evansi* and surra: a review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. *BioMed research international*.
Campigotto G. *et al*. (2015).- Experimental infection by *Trypanosoma evansi* in sheep: Occurrence of transplacental transmission and mice infection by parasite present in the colostrum and milk of infected ewes. *Veterinary parasitology*, **212**(3): 123-129.

There was detailed discussion regarding the conditions for a country or a zone to claim freedom after an outbreak of infection with *T. evansi*, especially when the control of the outbreak is based on the treatment of infected or serologically positive animals (Point 2.a.ii of Article 8.X.4). Indeed, trypanocide treatment may not always be curative, therefore, the Group recommended that parasitological screening and clinical observation of treated animals should be conducted monthly for at least 6 months to identify any persistence or relapse.

The proposed timelines and conditions for the recovery of a free status when the control of the outbreak is based on stamping out policy as described in Point 2.a.i of Article 8.X.4 are represented in Figure 1. Those described in Point 2.a.ii of Article 8.X.4 for a control based on a trypanocide treatments are represented in Figure 2.

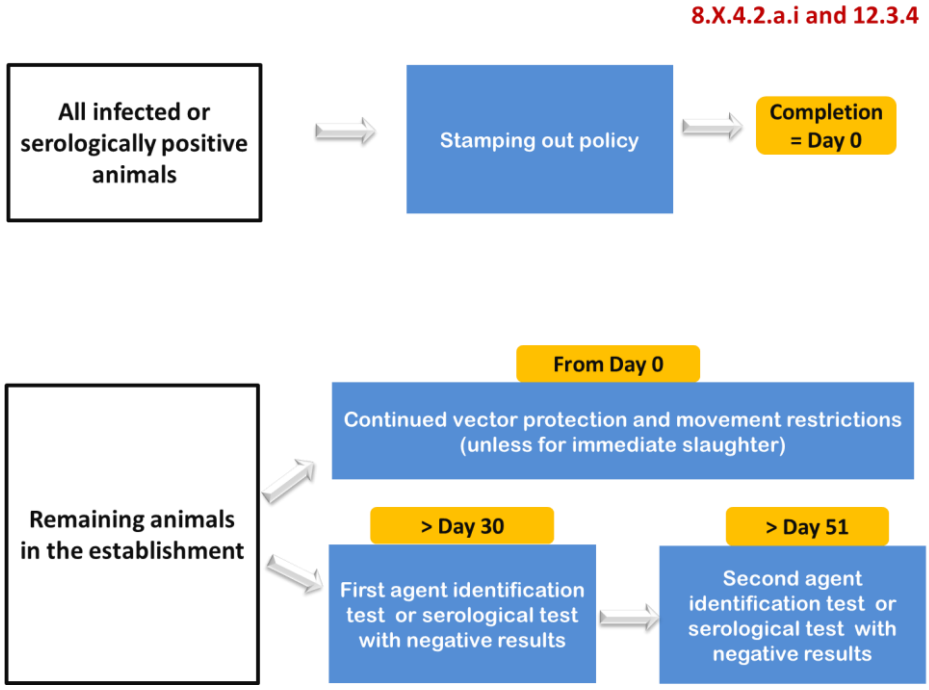

Figure 1. Recovery of a free status – Stamping out policy (Articles 8.X.4.2.a.i and 12.3.4)

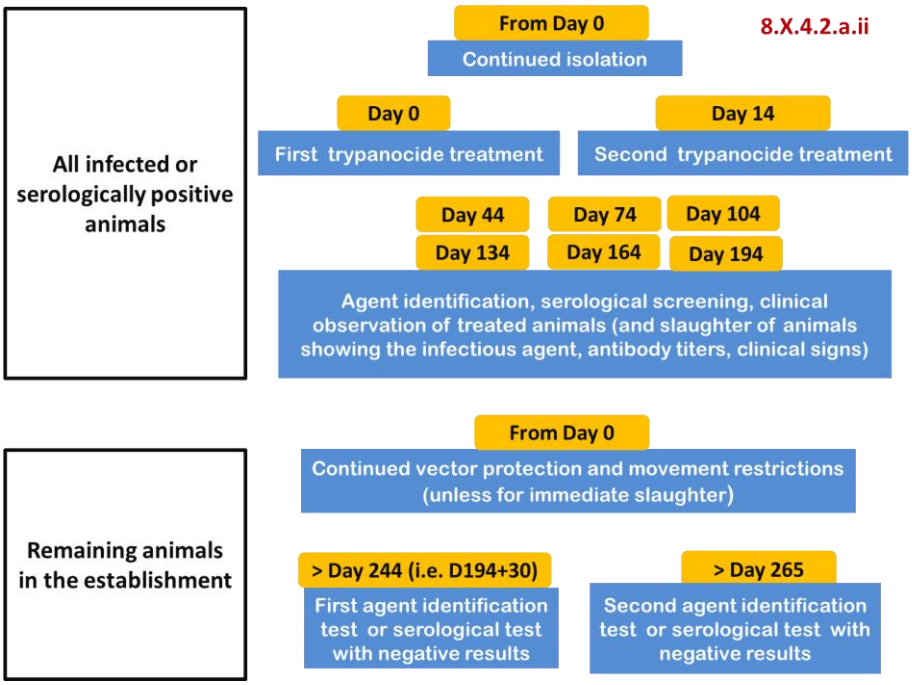

Figure 2. Recovery of a free status – Trypanocide treatment (Article 8.X.4.2.a.ii)

The Group agreed that after completion of the stamping out policy or the trypanocide treatment strategy, a specific surveillance for *T. evansi* should be conducted during a certain time period before the recovery of a free status can be declared (Point 3 of Article 8.X.4). The Group debated the duration of this surveillance period. The Group that met in 2015 recommended that it should be implemented for 2 years before the recovery of a free status (as stated in the *Meeting of the OIE Ad hoc Group on Equine Trypanosomoses – Paris, 21-23 July 2015)* or one year (as stated in the draft *Code* chapter annexed to this meeting report). Taking into consideration that the surveillance period would come in addition to the period during which the stamping out policy or the treatment strategy are applied (which would already take several months (i.e. about 2 months for a stamping out strategy and more than 8 months for a treatment strategy) and provide strong guarantees as to the status of the animal populations with regard to the infection with *T. evansi*) the Group determined that a surveillance period of six months would be acceptable for the purpose of this article.

- Since the Group had decided to include the possibility for a country or zone to claim freedom in specific animal species (see Article 8.X.3), **Article 8.X.5 (Recommendations for importation of camelids, carnivores, bovidae, pigs, cervids, elephants, lagomorphs, rodents and vampire bats)** was reorganized to include two sections: one for countries or zones free from infection in all host species (Point 2.a of Article 8.X.5) and one for countries or zones free in the imported species (Point 2.b of Article 8.X.5). To mitigate the risk of interspecific transmission, the Group agreed that animals imported from countries free in the imported species, but not in all other species should be isolated, protected against vectors and subjected to a diagnostic test prior to shipment. Animals imported from countries or zones not free in that specific species should be subjected to an additional test (two tests in total) (Point 2.c of Article 8.X.5).

- In regard to **Article 8.X.6 (Recommendations for importation of camelids, bovidae, pigs from an infected country or zone for direct slaughter**), the Group insisted on the notion of direct slaughter to mitigate the risk of transmission. The Group specified that the animals should be transported directly from the establishment of origin to the approved slaughterhouse/abattoir in a vector protected vehicle without coming into contact with other susceptible animals.

- **Article 8.X.7 (Recommendations for importation of semen)** was proposed as a novel article that the Group determined was necessary as there are reports of *T. evansi* present in the semen in rams.

  Again, since the Group had decided to include the possibility for a country or zone to claim freedom in specific animal species (see Article 8.X.3), Article 8.X.7 was modelled in different sections covering: freedom in all animal species (Point 2.a of Article 8.X.7); freedom in the relevant animal species (Point 2.b of Article 8.X.7); absence of freedom in the relevant animal species (Point 2.c of Article 8.X.7). Taking into consideration the risk of interspecies transmission, the Group recommended that the donor from a country or a zone free in the relevant species but not free in all species should be tested prior to entry in a semen collection facility. The Group recommended that in countries or zones not free in the relevant species, the donor males should be isolated and protected against vectors and should be tested twice prior to entry in a semen collection facility.

  The Group discussed the available assays to detect infection in semen and determined that microscopic evaluation would be unreliable and that, at this time, molecular assays (PCR) would be the most reliable assays. The Group recommended that testing of semen, including by molecular methods, should be further described in the *Terrestrial Manual*.

  The Group considered that there was not enough scientific evidence to support concerns about embryos specific to *T. evansi.* The Group therefore determined not to include specific recommendations for embryos in the draft chapter 8.X. Member Countries should refer to the provisions of the *Code* chapter 4.7. (Collection and processing of *in vivo* derived embryos from livestock and equids) in that regard.

6. Revised Chapter 12.3 (Infection with Trypanozoon in equids (dourine, equine surra))

Discussions on individual articles were as follows:

– The Group formatted **Article 12.3.1 (General Provisions)** similarly to Article 8.X.1 of the draft *Code* chapter 8.X. Importantly, a statement was added to justify the unification of *T. evansi*, *T. equiperdum*, and *T. brucei* infections in equids in a single chapter.

A statement was also added to indicate that the transmission of Trypanozoon can be mechanical, venereal, or tse-tse transmitted (*T. brucei*).

The Group noted the lack of data concerning the survival time for *T. brucei* and *T. equiperdum* in contaminated meat, and defined the duration of survival of Trypanozoon in contaminated meat listed in the General Provisions (72 hours) in reference to *T. evansi.*

A case definition was modelled after the one produced for the chapter 8.X. The Group discussed the timelines and what constitutes a confirmed case. It determined that, for the purposes of this *Code* chapter, a serologically positive equid showing clinical signs of infection with Trypanozoon or epidemiologically linked to a case should be considered infected.

The Group discussed the incubation period of infection with Trypanozoon in equids. Subclinical infections are possible, so the Group considered it difficult to set an incubation period. It was noted that it could take 60 days for a horse to seroconvert and become antibody positive. In theory, the incubation period could be as high as two years according to field data collected during the Italian dourine outbreak. This period and the consequences of this timeframe were discussed. The previous *Code* chapter indicated an incubation period of six months for dourine. The Group consensus was that it would be best to define the incubation period as 30 days as this represents the timeframe noted for experimental infections.

– In regard to **Article 12.3.2 (Safe Commodities)**, the Group discussed the similarities between the needs of this chapter and chapter 8.X. It was determined that wool, fibers and claws should be removed as this Chapter only references equids.

– The provisions of **Article 12.3.3 (Country or zone free from infection with Trypanozoon in equids)** were developed consistently with Article 8.X.3 (Country or zone free from infection with *T. evansi* in one or more animal species).

– **Article 12.3.4** (**Recovery of free status in equids)** was referenced from Article 8.X.4 (Recovery of a free status), however considering that treatment would only work for *T. evansi* and *T. equiperdum* if the parasite has not spread to the central nervous system, the option of treating infected or serologically positive equids was not included in Article 12.3.4. Consequently, only the option of applying a stamping out policy was left for a swift recovery of a free status from infection with Trypanozoon in equids. Alternatively, the recovery of the free status may also follow the path described in Article 12.3.3.

Considering the potential for subclinical cases of infections, the Group recommended that a disease-specific surveillance system should be conducted for at least 6 months after completion of the stamping out policy. The Group also highlighted the importance of compliance with the *Code* chapter 4.1 (General principles on identification and traceability of live animals) to ensure an adequate surveillance.

The proposed timelines and conditions for the recovery of a free status described in the Points 3 and 4 of Article 12.3.4 are illustrated in Figure 1.

– The Group modelled the recommendations of **Article 12.3.5** (**Recommendations for importation of equids**) after those defined in Article 8.X.5 (Recommendations for importation of camelids, carnivores, bovidae, pigs, cervids, elephants, lagomorphs, rodents and vampire bats).

– With regard to **Article 12.3.6 (Recommendations for the temporary importation of horses for competition purposes**), the Group harmonised the conditions applicable to horses imported from a country or a zone free from infection with Trypanozoon in equids and not free from infection with *T. evansi* in all other species and those applicable to horses imported from a country or a zone not free from infection with Trypanozoon in equids. The rationale for that harmonisation is a presumed lower likelihood of transmission of the infection by horses imported on a temporary basis for competition purposes due to: (i) the shorter duration of the stay in the importing country, and (ii) the limited contacts with the local animal populations. However, the Group insisted that importing countries should consider the expected inherent risk associated with the horses imported under these conditions from a country or a zone not free from infection with Trypanozoon in equids and should keep them separated from the domestic population.

– The Group modelled the recommendations of **Article 12.3.7 (Recommendations for importation of equids from a country or zone not free from infection with Trypanozoon in equids for direct *slaughter***) after those defined in Article 8.X.6 (Recommendations for importation of camelids, bovidae, pigs from an infected country or zone for direct slaughter), and the recommendations of Article 12.3.8 (Recommendations for importation of semen) after those defined in Article 8.X.7 (Recommendations for importation of semen). The Group consulted Chapters 4.5 and 4.6 for recommendations regarding semen collection and processing and noted that Chapter 4.6 does not address equids, but only references bovine, porcine, and small ruminant and it should therefore not be cross-referenced in Chapter 12.3.

7. Recommendations for the revision of the *Manual* Chapters

The Group expressed the need for revising the chapters 2.1.21. (*Trypanosoma evansi* infections (including surra)) and 2.5.3. (Dourine) of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (hereafter the *Manua*l).

The Group was of the opinion that the *Manual* chapters should be aligned with the proposed scope of the *Code* chapters. Therefore the Group suggested the Scientific Commission to refer to the Biological Standards Commission consideration's on whether a *Manual* chapter on infections with Trypanozoon in equids should replace the current *Manual* chapter 2.5.3. on Dourine -on the model of the *Manual* chapter 2.1.4. [Brucellosis (*Brucella abortus, B. melitensis* and *B. suis*)]-.

The Group reviewed the following recommendations for revisions to the OIE *Manual* listed in the report from the *Meeting of the OIE Ad Hoc Group on Equine Trypanosomoses – Paris, 21-23 July2015*:

- "The Surra *Manua*l chapter should specify that in case of detection of *T. evansi* the agent identification test should include a PCR in order to exclude as a first step *T. brucei*;
- The Surra *Manual* chapter should contain also a "fit for purpose test" table, as already included in the Dourine chapter;
- The Dourine *Manual* chapter should be aligned to the *Code* chapter regarding the use of the term "breeding animals";
- Include in the *Manual* a statement that treatment is possible for both diseases, but only for the bloodstream form, not once the parasite has crossed the cerebrospinal fluid barrier".

The Group disagreed with the statement suggesting that "the Surra *Manual* Chapter should specify that in case of detection of *T. evansi* the agent identification test should include a PCR in order to exclude as a first step *T. brucei*" on the basis that such a distinction is not systematically necessary: (i) if an animal is found with trypanosomosis in a non-tsetse endemic country, *T. brucei* would not be included on the differential diagnosis list; (ii) the treatment of the animal would be the same if it is infected by *T. evansi* or by *T. brucei*. The Group therefore concluded that this recommendation would be irrelevant outside of the African continent where tsetse flies are endemic. The Group recommended that a battery or panel of PCR assays should be conducted to distinguish *T. evansi*, *T. equiperdum*, and *T. brucei*.

The Group unanimously supported the statement that "The Surra *Manual* Chapter should contain also a "fit for purpose test" table, as already included in the Dourine chapter".

In addition, the Group listed a number of other issues that would need to be addressed in the *Manual* chapters and recommended to forward them to the *Biological Standards Commission*:

- the occurrence of human cases of infection with *T. evansi* (as evocated in the section 5 of this report, Article 8.X.1);
- the pathogenicity of *T. evansi* in the different host species;
- the reasons why more than one test might be required to establish an individual health status (Articles 8.X.4, 8.X5, 8.X.7, 12.3.4, 12.3.5, 12.3.8);
- the efficacy of trypanocide treatments (including the penetration of drugs into tissues and the central nervous system and the use of serology to monitor efficacy of treatment's);
- criteria for the genetic characterization of the trypanosome species;
- molecular methods for testing semen.

In addition, the Group expressed needs for:

- the validation of assays for the detection of *T. evansi* in the different host species;
- the characteristics of the PCR assays (sensitivity, specificity);
- the definition of reference strains;
- the definition of diagnostic pathways.

8. Adoption of the report

The Group reviewed and amended electronically the draft report provided by the rapporteur. The Group agreed that the report captured the discussions.

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…/Appendices

Appendix I

OIE AD HOC GROUP ON EQUINE TRYPANOSOMOSES

Paris, 14-16 June 2016

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Terms of Reference

On the basis of the preliminary work conducted by the OIE ad hoc Group on equine trypanosomoses conveyed in Paris in July 2015, further develop a *Code* Chapter on Surra and revise the *Code* Chapter on Dourine.

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Agenda

1. Opening
2. Adoption of the agenda and appointment of chairperson and rapporteur
3. Presentation of the comments of members of the Scientific Commission and Code Commission on the report of the previous ad hoc Group
4. Revision of the scope of the *Code* chapters
5. Chapter 8.X. (Infection with Trypanosoma evansi – non equine surra)
6. Chapter 12.3 (Infection with Trypanozoon in equids (dourine, equine surra))
7. Recommendations for the revision of the *Manual* Chapters

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Appendix II

OIE AD HOC GROUP ON EQUINE TRYPANOSOMOSES

Paris, 14-16 June 2016

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