

OPINION 04-2016

Subject:

Control of dourine in equids

(SciCom 2015/14)

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Key terms:

Dourine, Equids, Horses, Trypanosoma

Sleutelwoorden:

Dourine, Paardachtigen, Paarden, Trypanosoma

Mots-clés:

Dourine, Equidae, Chevaux, Trypanosomes

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Executive summary

Background & Terms of reference

Dourine is a serious acute or chronic venereal infectious parasitic disease of horses and other equids. It is an officially notifiable disease for which European legislation establishes requirements with regard to intra-Community trade. The disease is considered to be eradicated in Europe, but in 2011 and 2012 several outbreaks occurred in Italy.

Dourine is an OIE listed venereal disease of equids caused by the 'so called' protozoan tissue parasite *Trypanosoma equiperdum*. In the scientific literature controversies exist regarding the etiology of dourine and the classification of *T. equiperdum* because of the genetic overlap that occurs with *T. evansi* and *T. brucei brucei*. The existence of *T. equiperdum* as a separate strain is questioned as most of the isolated strains appear to cluster molecularly with *T. evansi*. Molecular methods do not enable consistent discrimination between *T. evansi* and *T. equiperdum* strains.

Generally it is believed that transmission of *T. equiperdum* occurs during mating or by artificial insemination with infected semen. *T. evansi* can be transmitted very easily by haematophagous insects. Transmission of infection between animals can occur via venereal (*T. equiperdum*) and mechanical way (*T. evansi*).

In order to prepare a new legislation in regard to the control of dourine in equids a number of specific questions are asked to the Scientific Committee:

1. What is a (confirmed) case of dourine?
2. Is dourine a zoonosis?
3. Which diagnostic procedure should be used to confirm a case of dourine?
4. After exposure, what is the incubation time during which diagnostic tests can give a false negative result?
5. When can be accepted that a horse is suspected or confirmed infected by dourine?
6. Which diagnostic procedure should be applied to confirm absence of the parasite in semen?
7. Can tissues other than sperm be infected?
8. Which measures are recommended to limit the spread of the disease after identification of a positive horse?
9. Should euthanasia be systematically applied to infected horses?
10. Should castration be considered as a control measure?
11. Which control measures are recommended for horses having had contact with an infected animal or an animal suspected to be infected? Which kind of contacts should be accounted for?

Methodology

This opinion is based on evidence from scientific literature and on expert opinion.

Answers to the questions

The Scientific Committee supports the definition adopted by OIE of a (confirmed) case of dourine but is of the opinion that the clinical picture corresponding to dourine can be caused by *T. equiperdum* as well as by *T. evansi*. This means that the disease is not only a venereal transmittable disease but that the agent can also be transmitted mechanically (by invertebrate vectors). The confirmation of dourine should be based on the clinical picture, the existence of epidemiological risk factors and a positive laboratory test such as a serological complement fixation test (CFT), an indirect immunofluorescence test, an ELISA or a molecular test.

A horse is suspected to be infected by dourine if epidemiological risk factors apply such as 'originating from an endemically infected region' or if clinical symptoms, pathognomonic for dourine, are present. The Scientific Committee is of the opinion that consecutive complement fixation tests should be performed at minimum 2 weeks interval in order to confirm a suspicious case of dourine. Dourine is not a zoonosis.

According to OIE, the incubation period of dourine is very variable and may vary from 1 week to a few months. The Scientific Committee is of the opinion that during incubation time false negative diagnostic tests can occur up to 4 weeks after infection. Antibodies are expected to be detectable within 3 weeks after infection. Parasite detection and molecular tests may stay negative much longer. This is the reason why the antibody detection with CFT or similar is the test of choice for screening.

T. equiperdum is a widely distributed parasite in the infected organism (semen, eggs, foetus, milk, ...). The Scientific Committee recommends to use a molecular test to investigate semen for presence or absence of the parasite.

Conclusion

The Scientific Committee is of the opinion that the risk of introduction of dourine in Belgium is very low but that vigilance is certainly warranted given the globalization of trade in semen and intercontinental travelling of horses. Transmission of infection between animals can occur via venereal (*T. equiperdum*) and mechanical way (*T. evansi*). The diagnosis of dourine is a challenge and is based on a combination of presence of clinical symptoms, results of serological tests and presence of an epidemiological link with the disease. Given the fact that Belgium is free from dourine it is recommended to slaughter or euthanize a confirmed case as soon as possible to limit the spread of the disease. Castration is not a valuable alternative measure as infection can be transmitted by horseflies.

Samenvatting

Achtergrond & Referentietermen

Dourine is een ernstige acute of chronische besmettelijke venerische parasitaire ziekte van paarden en andere paardachtigen. Het is een officieel aangifteplichtige ziekte waarvoor Europese wetgeving eisen stelt met betrekking tot het intracommunautaire handelsverkeer. De ziekte wordt geacht te zijn uitgeroeid in Europa, maar in 2011 en 2012 hebben zich een aantal uitbraken voorgedaan in Italië.

Dourine is een venerische ziekte van paardachtigen op de OIE-lijst, die wordt veroorzaakt door de 'zogenaamde' protozoaire weefselparasiet *Trypanosoma equiperdum*. In de wetenschappelijke literatuur bestaat controversie met betrekking tot de etiologie van dourine en de indeling van *T. equiperdum* vanwege de overlapping die zich voordoet met *T. evansi* en *T. brucei brucei*. Het bestaan van *T. equiperdum* als afzonderlijke stam wordt in vraag gesteld aangezien de meeste geïsoleerde stammen moleculair lijken te clusteren met *T. evansi*. Moleculaire methoden zijn niet in staat om op consistente wijze een onderscheid te maken tussen *T. evansi* en *T. equiperdum* stammen.

Algemeen wordt aangenomen dat de overdracht van *T. equiperdum* optreedt tijdens de dek of door kunstmatige inseminatie met besmet sperma. *T. evansi* kan heel gemakkelijk worden overgedragen door haematofage insecten. Overdracht van infectie tussen dieren kan optreden langs venerische (*T. equiperdum*) of mechanische weg (*T. evansi*).

Om een nieuwe wetgeving met betrekking tot de controle van dourine bij paardachtigen voor te bereiden worden een aantal specifieke vragen gesteld aan het Wetenschappelijk Comité:

1. Wat is een (bevestigd) geval van dourine?
2. Is dourine een zoönose?
3. Welke diagnostische procedure moeten worden gebruikt om een geval van dourine te bevestigen?
4. Hoelang bedraagt, na blootstelling, de incubatietijd gedurende dewelke diagnostische tests een vals negatief resultaat kunnen geven?
5. Wanneer kan worden aangenomen dat een paard verdacht of bevestigd besmet is met dourine?
6. Welke diagnostische procedure moet worden toegepast om de afwezigheid van de parasiet in sperma te bevestigen?
7. Kunnen andere weefsels dan zaadcellen worden besmet?
8. Welke maatregelen worden aanbevolen om de verspreiding van de ziekte te beperken na de identificatie van een positief geval ?
9. Moet euthanasie systematisch worden toegepast op besmette paarden?
10. Kan castratie worden beschouwd als een controlemaatregel?
11. Welke controlemaatregelen worden aanbevolen voor paarden die contact hebben gehad met een besmet dier of voor een verdacht besmet dier? Met welke soort contacten dient rekening te worden gehouden?

Methodologie

Dit advies is gebaseerd op gegevens uit de wetenschappelijke literatuur en op expert opinie.

Antwoorden op de gestelde vragen

Het Wetenschappelijk Comité steunt de door de OIE aangenomen definitie van een (bevestigd) geval van dourine, maar is van mening dat het klinisch beeld dat overeenkomt met dourine zowel kan worden veroorzaakt door *T. equiperdum* als door *T. evansi*. Dit betekent dat de ziekte niet alleen een venerisch overdraagbare ziekte is, maar dat het agens ook mechanisch kan worden overgedragen

(door ongewervelde vectoren). De bevestiging van dourine moet worden gebaseerd op het klinisch beeld, het bestaan van epidemiologische risicofactoren en op een positieve laboratoriumtest zoals een serologische complementbindingsreactie (CFT-test), een indirecte fluorescentie test, een Elisa of een moleculaire test.

Een paard wordt verdacht van dourine als er epidemiologische risicofactoren van toepassing zijn, zoals 'afkomstig uit een endemisch besmet gebied' of indien pathognomonische symptomen voor dourine aanwezig zijn. Het Wetenschappelijk Comité is van mening dat de opeenvolgende CFT tests moeten worden uitgevoerd met minimum 2 weken interval om een verdacht geval van dourine te bevestigen. Dourine is geen zoönose.

Volgens de OIE, is de incubatietijd van dourine zeer variabel en kan variëren van 1 week tot een paar maanden. Het Wetenschappelijk Comité is van mening dat tijdens de incubatietijd vals-negatieve resultaten van diagnostische tests kunnen voorkomen tot 4 weken na de infectie. Antilichamen zijn detecteerbaar binnen de 3 weken na infectie. De detectie van de parasiet en moleculaire testen kunnen veel langer negatief blijven. Dit is de reden waarom de antilichaamdetectie met de CFT-test of met gelijkaardige testen de voorkeur wegdragen voor screening doeleinden.

T. equiperdum is een weefselparasiet (sperma, eieren, foetus, melk, ...). Het Wetenschappelijk Comité raadt aan om een moleculaire test te gebruiken om de aan- of afwezigheid van de parasiet in sperma na te gaan.

Conclusie

Het Wetenschappelijk Comité is van mening dat het risico van insleep van dourine in België zeer laag is, maar dat waakzaamheid zeker gerechtvaardigd is, gezien de globalisering van de handel in sperma en de intercontinentale verplaatsingen van paarden. Overdracht van infectie tussen dieren kan optreden langs venerische (*T. equiperdum*) en mechanische weg (*T. evansi*). De diagnose van dourine is een uitdaging en is gebaseerd op een combinatie van de aanwezigheid van klinische symptomen, de resultaten van serologische tests en de aanwezigheid van een epidemiologisch verband met de ziekte. Gezien België vrij is van dourine is het raadzaam om een bevestigd geval zo snel mogelijk te slachten of te euthanaseren om de verspreiding van de ziekte te beperken. Castratie is geen waardevol alternatieve maatregel aangezien de infectie door steekvliegen kan worden overgedragen.

Résumé

Contexte & cadre de référence

La dourine est une maladie vénérienne parasitaire contagieuse grave, aiguë ou chronique, des chevaux et autres équidés. C'est une maladie à déclaration obligatoire pour laquelle la réglementation Européenne impose des exigences en ce qui concerne le commerce intra-communautaire. La maladie est considérée comme éradiquée en Europe, mais en 2011 et 2012, un certain nombre de foyers sont apparus en Italie.

La dourine est une maladie vénérienne équine qui se trouve sur la liste de l'OIE et serait provoquée par le parasite protozoaire tissulaire dénommé *Trypanosoma equiperdum*. En effet, dans la littérature scientifique, il y a des controverses sur l'étiologie de la dourine et la classification de *T. equiperdum* en raison du croisement avec *T. evansi* et *T. brucei brucei*. L'existence de *T. equiperdum* comme une souche distincte est remise en question comme la plupart des souches isolées se regroupent avec *T. evansi*. Les méthodes moléculaires ne peuvent pas faire une claire distinction entre les souches *T. evansi* et *T. equiperdum*.

Il est généralement admis que la transmission de *T. equiperdum* se produit lors de la saillie ou par insémination artificielle avec la semence infectée. *T. evansi* peut être transféré très facilement par des insectes hématophages. La transmission de l'infection entre les animaux peut se produire par voie sexuelle (*T. equiperdum*) ou par voie mécanique (*T. evansi*).

Afin de préparer une nouvelle législation concernant le contrôle de la dourine chez les équidés un certain nombre de questions sont posées au Comité scientifique:

1. Qu'est-ce que un cas (confirmé) de dourine?
2. Est-ce-que la dourine est une zoonose?
3. Quelle est la procédure de diagnostic qui doit être utilisée pour confirmer un cas de dourine?
4. Après l'exposition, quelle est la période d'incubation pendant laquelle les tests de diagnostic peuvent donner un résultat faussement négatif?
5. Quand peut-on supposer qu'un cheval est suspect ou est confirmé comme étant infecté par la dourine?
6. Quelle est la procédure de diagnostic qui doit être utilisée pour confirmer l'absence du parasite dans les semences?
7. Est-ce que d'autres tissus que la semence peuvent être infectés?
8. Quelles sont les mesures recommandées pour réduire la propagation de la maladie à partir de l'identification d'un cas positif?
9. L'euthanasie doit-elle être systématiquement appliquée aux chevaux infectés?
10. La castration peut-elle être considérée comme une mesure de contrôle?
11. Quelles mesures de contrôle sont recommandées pour les chevaux qui ont été en contact avec un animal infecté ou avec un animal suspect affecté? Quels contacts devraient être pris en compte?

Méthodologie

Cette opinion est fondée sur les données de la littérature scientifique et sur une opinion d'experts.

Les réponses aux questions

Le Comité scientifique soutient la définition adoptée par l'OIE d'un cas de dourine, mais estime que le tableau clinique correspondant à la dourine peut être causé par *T. equiperdum* comme par *T. evansi*. Cela signifie que la maladie est non seulement une maladie à transmission sexuelle, mais que l'agent peut également être transmis par voie mécanique (par des vecteurs invertébrés). La confirmation de

dourine devrait être basée sur le tableau clinique, sur l'existence de facteurs de risque épidémiologiques et sur un test positif, comme un test sérologique de fixation du complément (test CFT), le dosage par immunofluorescence indirecte ou par un test ELISA ou par un test moléculaire.

Un cheval est soupçonné d'être infecté par la dourine si des facteurs de risque épidémiologiques sont applicables comme 'provenant d'une zone endémique' ou si les symptômes pathognomoniques de la dourine sont présents. Le Comité scientifique estime que les tests CFT successifs doivent être effectués avec un minimum de 2 semaines d'intervalle pour confirmer un cas suspect de dourine. La dourine n'est pas une zoonose.

Selon l'OIE, la période d'incubation de la dourine est très variable et peut varier d'une semaine à quelques mois. Le Comité scientifique estime que, pendant la période d'incubation, des résultats faussement négatifs peuvent se produire jusqu'à 4 semaines après infection. Les anticorps sont détectables dans les 3 semaines après l'infection. La détection des parasites et les tests moléculaires peuvent rester négatifs pendant beaucoup plus longtemps. Ceci est la raison pour laquelle la détection des anticorps avec le test CFT ou avec un test similaire est préférée à des fins de dépistage.

T. equiperdum est un parasite largement distribué dans l'organisme infecté (on le retrouve dans le sperme, les œufs, les fœtus, le lait, ...). Le Comité scientifique recommande d'utiliser un test moléculaire pour détecter la présence ou l'absence du parasite dans la semence.

Conclusion

Le Comité scientifique est d'avis que le risque d'introduction de la dourine en Belgique est très faible, mais que la vigilance est certainement justifiée, compte tenu de la mondialisation des échanges dans les semences et les mouvements intercontinentaux de chevaux. La transmission de l'infection entre les animaux peut se produire par voie sexuelle (*T. equiperdum*) et par voie mécanique (*T. evansi*). Le diagnostic de la dourine reste un défi et est basé sur une combinaison de la présence de symptômes cliniques, des résultats des tests sérologiques et de la présence d'un lien épidémiologique avec la maladie. Étant donné que la Belgique est indemne de la dourine, il est conseillé d'abattre ou d'euthanasier tout cas confirmé le plus tôt possible pour limiter la propagation de la maladie. La castration n'est pas une mesure alternative intéressante puisque l'infection peut être transmise par les insectes piqueurs.

1. Terms of reference

1.1. Questions

In order to prepare a new legislation in regard to the control of dourine in equids a number of specific questions are asked to the Scientific Committee. From the viewpoint of pursuing clarity and intelligibility of this opinion the original list of questions was, with the agreement of the questioner, extended to the following set :

1. What is a (confirmed) case of dourine?
2. Is dourine a zoonosis?
3. Which diagnostic procedure should be used to confirm a case of dourine?
4. After exposure, what is the incubation time during which diagnostic tests can give a false negative result?
5. When can be accepted that a horse is suspected or confirmed infected by dourine?
6. Which diagnostic procedure should be applied to confirm absence of the parasite in semen?
7. Can tissues other than sperm be infected?
8. Which measures are recommended to limit the spread of the disease after identification of a positive horse?
9. Should euthanasia be systematically applied to infected horses?
10. Should castration be considered as a control measure?
11. Which control measures are recommended for horses having had contact with an infected animal or an animal suspected to be infected? Which kind of contacts should be accounted for?

1.2. Legal provisions

Dourine is a compulsory notifiable disease affecting horses as stipulated:

- by the royal decree of 3 February 2014 designating the animal diseases covered by the application of chapter III of the animal health law of 24 March 1987 and regulating the obligatory declaration,
- by Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community and
- by Council Directive 90/426 of 26 June 1990 on animal health conditions governing the movement and import from third countries of equidae.

The royal decree of 31 December 1921 in regard to measures of veterinary police to control dourine in equids has been repealed by the royal decree of 3 February 2014 designating the animal diseases covered by the application of Chapter III of the animal health law of 24 March 1987 and regulating obligatory declaration.

1.3. Methodology

This opinion is based on evidence from scientific literature and on expert opinion.

2. Abbreviations

- CATT: card agglutination test for trypanosomiasis
- CFSPH: Center for Food Security & Public Health (Iowa State University)
- CFT: complement fixation test
- DEFRA: Department for Environment, Food & Rural Affairs of the United Kingdom
- ELISA: enzyme-linked immunosorbent assay
- IFAT : indirect fluorescent antibody test

- OIE: 'Office International des Epizooties' (World Organization for Animal Health)
- PCR: polymerase chain reaction

Considering the discussions during the workgroup meeting of 22nd October 2015 and during the plenary session of the Scientific Committee on 18th March 2016,

the Scientific Committee gives the following opinion:

3. Introduction

Dourine is an officially notifiable disease for which European legislation establishes requirements with regard to intra-Community trade. Dourine is a disease that is also regulated by the OIE. The disease is considered to be eradicated in Europe, but in 2011 and 2012 several outbreaks occurred in Italy (Pascucci et al, 2013).

The Belgian authorities want to prepare for possible outbreaks of dourine and wish to adapt the necessary legislation to control these outbreaks. The questions addressed to the Scientific Committee aim to provide the risk manager with key information to prepare a new legislation.

4. Risk assessment

4.1. Actual knowledge in regard to dourine in equids

4.1.1. Hazard identification & characterization

The disease

Equine trypanosomosis can be caused by different species of *Trypanosoma*: *Trypanosoma equiperdum* (dourine), *Trypanosoma evansi* (surra), *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax* (nagana). The clinical signs associated with these parasites are similar.

According to the Center for Food Security and Public Health (Iowa State University, 2015) and the OIE Terrestrial Manual (2013) dourine is a serious acute or chronic venereal infectious disease of horses and other equids. Dourine is an OIE list venereal disease of equids caused by the 'so called' protozoan tissue parasite *Trypanosoma equiperdum* (Doflein, 1901). In scientific literature controversy exists in regard to the characterization of the *Trypanosoma* strain, which has historically been associated with dourine.

The disease is characterized by a pathognomonic clinical picture consisting of genital edema with mucopurulent discharge, cutaneous plaques, neurological signs and progressive deterioration. The course of the disease may vary with the strain. In some horses a relatively mild disease persists for years with periodic exacerbations; in other horses an acute form lasts only 1 to 2 months.

Occurrence of the disease

According to OIE (2015) dourine occurs in most of Asia, northern and southern Africa, Russia, parts of the Middle East, South America and southeastern Europe. In 2011 and 2012 several outbreaks occurred in Italy (OIE). The outbreak of dourine in Italy was probably caused by illegal import of horses for slaughtering of which some were eventually not slaughtered or had the chance to mate before they were slaughtered.

Since then only an outbreak in Botswana (2015-2016) is reported by OIE-WAHIS (verified on 16th February 2016). Belgium is officially free from dourine according to the rules of OIE.

The parasite

Trypanosomes are unicellular parasites causing disease in humans and animals. Trypanosomes comprise a wide group of parasites of vertebrates usually transmitted by haematophagous arthropods. *T. congolense*, *T. vivax*, *T. equiperdum*, *T. brucei* and *T. evansi* may cause disease in livestock and belong to the Salivaria section. The latter three species belong to the same Trypanozoon subgenus.

In the scientific literature (Claes et al., 2005; Li et al., 2006, Shaw 2006, Claes et al., 2006) controversies exist regarding the etiology of dourine and the classification of *T. equiperdum* because of the genetic overlap that occurs with *T. evansi* and *T. brucei brucei*. It is accepted that *T. equiperdum*, *T. b. brucei* and *T. evansi* belong to the subgenus *Trypanozoon* but Claes et al. (2005) drew the attention to the difficulties to associate the clinical condition of dourine with *T. equiperdum* that is supposed to cause the disease. According to Claes et al. (2005) dourine is a disease caused by specific host immune responses to a *T. equiperdum* or *T. evansi* infection.

Molecular methods do not enable consistent discrimination between *T. evansi* and *T. equiperdum* strains (Claes et al., 2005). The mitochondrial DNA of *T. equiperdum* contains maxi-circles. This is not the case for *T. evansi* (Li et al., 2007).

The existence of *T. equiperdum* as a separate strain is questioned as officially no strain has any more been isolated since 1981 and most of the previously isolated strains appear to cluster molecularly with *T. evansi* (Hagos et al., 2010). It has to be said that since 2010 new isolations of *T. equiperdum*, as defined by molecular differentiating techniques, have been done in Ethiopia and Venezuela. Definitive categorization of dourine is pending (OIE, 2015).

Transmission of the disease

According to OIE (2013) dourine (*T. equiperdum*) is the only type of trypanosomosis that is not transmitted by an invertebrate vector. However experimental infections indicate that mechanical transmission by haematophagous arthropods cannot be excluded as a possible infection route (Van Den Bossche et al., 2004). Generally it is believed that transmission of *T. equiperdum* occurs during mating (Stephen, 1986) or by artificial insemination with infected semen.

According to DEFRA (2011) transmission from stallions to mares is more common, but mares can also transmit the disease to stallions. *T. equiperdum* can be found in the vaginal secretions of infected mares and the seminal fluid, mucous exudates of the penis and sheath of stallions. Periodically the parasite seems to disappear from the genital tract and the animal becomes non-infectious for weeks to months. Transmission is most likely early in the disease process. Male donkeys can be asymptomatic carriers and sexually immature animals that become infected can transmit the organism when they mature. Rarely, infected mares pass the infection to their foals, possibly before

birth or through colostrum and milk. Infection may also occur via mucous membranes such as the conjunctivae. Foals may transmit the disease when they are sexually mature.

T. evansi can be transmitted very easily by haematophagous insects.

Diagnosis

Dourine is usually diagnosed by clinical evidence and serology. A definitive diagnosis depends on the identification of the parasite, which is not always possible. The prescribed test for international trade is the complement fixation test (CFT). This test does not differentiate clearly between *T. equiperdum* and other Trypanosoma such as *T. brucei brucei* and *T. evansi* (Robinson, 1926) as cross-reactions might occur (OIE, 2013). Uninfected equids often give inconsistent or nonspecific reactions (OIE, 2013). In the case of anti-complementary sera, the indirect fluorescent antibody test can be used (OIE, 2013). Other tests have been used such as the enzyme linked immunosorbent assays, the agar gel immunodiffusion test (AGID), the radioimmunoassay, the counter-current immunoelectrophoresis test. Cauchard et al. (2014) organized inter-laboratory ring trials to evaluate the serological methods for dourine diagnosis. Their study confirmed the reliability of the complement fixation test (CFT) and highlighted its interlaboratory reproducibility for known *T. equiperdum* positive and negative sera. However the reproducibility was less good for sera positive for *T. evansi* or of unknown status.

4.1.2. [Entry assessment](#)

Horses and horse semen imported from dourine endemic areas could be infected with the pathogenic agent(s) responsible for dourine.

Imports of semen especially from stallions originating from dourine endemic areas represent a risk of introducing dourine.

Horses temporarily imported for competition and not for breeding represent a minimal risk of introducing dourine as long as mechanical transmission can be prevented.

4.1.3. [Exposure assessment](#)

Dourine is mostly spread by venereal contact although mechanical transmission cannot be excluded. *T. equiperdum* is transmitted by venereal route. Trypanosoma evansi is transmitted mechanically through biting horseflies. There are also reports of transmission of *T. equiperdum* to foals during birth and through maternal milk (Brun et al., 1998). Horses imported for breeding present a risk. However few horses in Belgium are bred via natural cover. Most (sport)horses are bred via artificial insemination.

4.1.4. [Consequence assessment](#)

Introducing dourine may have serious consequences and especially for those animals having had contact or an epidemiological link with the index case. As there is no effective treatment for infected horses temporarily quarantine of suspected animals and early eradication are the usually applied measures by the authorities. As dourine affects only equids, the consequences are confined to the equine sector.

4.1.5. [Risk estimation](#)

Introduction of infected horses or semen from endemic areas could lead to the introduction and establishment of dourine.

The Scientific Committee is of the opinion that the probability of entry and establishment of dourine into Belgium is very low because:

- of the compulsory serological testing (CFT) of horses prior to import in Belgium from endemic countries,
- of lack of contact between competition horses with horses at risk of dourine,
- most (sport) horses are bred via artificial insemination.

4.2. *Uncertainties*

The major uncertainties in this dossier are related to the controversy in scientific literature in regard to the identification/characterization of the pathogenic agent of dourine (*T. equiperdum* versus *T. evansi*) and the poor specificity of serological tests (cross-reactions with other Trypanosoma).

4.3. *Answers to the questions formulated in the terms of reference*

4.3.1. What is a (confirmed) case of dourine?

According to OIE (2013) a confirmed case of dourine is defined as an animal having a positive result with CFT or IFAT or PCR **and** (i) showing clinical signs compatible with dourine **or** (ii) showing an increase in serological CFT titer in two consecutive tests **or** (iii) is epidemiologically linked with a confirmed case of dourine.

The Scientific Committee supports the definition adopted by OIE of a (confirmed) case of dourine but is of the opinion that the clinical picture corresponding to dourine can be caused by *T. equiperdum* as well as by *T. evansi*. This means that the disease is not only a venereal transmittable disease but that the agent can also be transmitted mechanically (by invertebrate vectors).

4.3.2. Is dourine a zoonosis?

No human cases have been reported (OIE, 2013). There is no evidence that *T. equiperdum* can infect humans (CFSPH, 2015). The Scientific Committee is of the opinion that dourine is not a zoonosis.

4.3.3. Which diagnostic procedure should be used to confirm a case of dourine?

The confirmation of dourine should be based on the clinical picture, the existence of epidemiological risk factors and a positive laboratory test such as:

- a serological complement fixation test (CFT),
- a serological indirect fluorescence test (IFAT),
- a serological ELISA test,
- a molecular test.

The Scientific Committee is of the opinion that consecutive CFT tests should be performed at minimum 2 weeks interval in order to confirm a suspicious case of dourine. It has to be known that a negative PCR test is inconclusive as the PCR test is not 100% sensitive.

It is proposed that in legislation the term 'molecular test' be used instead of 'PCR test'.

4.3.4. After exposure, what is the incubation time during which diagnostic tests can give a false negative result?

According to OIE, the incubation period of dourine is very variable and may vary from 1 week to a few months. In the OIE Terrestrial Animal Health Code, the incubation period for dourine is set at 6 months.

In experimental infections in horses, antibodies against *T. evansi* were detected by CATT after 32 days. CATT has been developed for *T. evansi* but weak cross-reactions with *T. equiperdum* may occur (Hagos et al., 2010).

The Scientific Committee is of the opinion that during incubation time false negative diagnostic tests can occur up to 4 weeks after infection. Antibodies are expected to be detectable within 3 weeks after infection (see also section 4.3.8.). Parasite detection and molecular tests may stay negative much longer. This is the reason why the antibody detection with CFT or similar is the test of choice for screening.

4.3.5. When can be accepted that a horse is suspected or confirmed infected by dourine?

According to the Scientific Committee a horse is suspected to be infected by dourine if epidemiological risk factors apply such as 'originating from an endemically infected region' or if clinical symptoms pathognomonic for dourine are present.

A horse is confirmed infected by dourine if the diagnostic conditions of OIE are met (cfr. 4.3.1.).

4.3.6. Which diagnostic procedure should be applied to confirm absence of the parasite in semen?

The Scientific Committee recommends to use a molecular test to investigate semen for presence or absence of the parasite. In case of a negative molecular test on semen and a negative serological CFT test it can be concluded that the parasite is absent in semen.

In case the stallion is not alive anymore, only a molecular test can be performed on frozen semen. In case of a negative result, the semen cannot be guaranteed parasite-free. In case of a positive result The Scientific Committee recommends that the stock of frozen sperm be destroyed.

It cannot be guaranteed that semen from a CFT positive stallion is not infected.

4.3.7. Can tissues other than sperm be infected?

T. equiperdum is a tissue parasite. The Scientific Committee is of the opinion that in animals with dourine all tissues can be infectious (eggs, foetus, milk, ...).

4.3.8. Which measures are recommended to limit the spread of the disease after identification of a positive horse?

T. equiperdum is transmitted by venereal route. *T. evansi* is transmitted mechanically through biting horseflies. According to the Scientific Committee, after careful consideration of the available scientific literature and expert opinion, both *T. equiperdum* and *T. evansi* may cause dourine.

The Scientific Committee recommends to euthanize a confirmed case of dourine as soon as possible. Latently infected animals (without clinical symptoms) can also be slaughtered provided that guarantees are given that it is performed shortly after the diagnosis and that the risk for transmission

of the infection via arthropods during the interim period and the transport to the slaughterhouse is under complete control.

Animals which have had contact with a confirmed case of dourine are considered to be suspected cases of dourine because of the existence of an epidemiological link. The Scientific Committee recommends to take the following measures in regard to suspected cases:

- to serologically test the animal twice with 25 days interval (CFT test),
- to put the animal in quarantine till the result of the 2nd CFT test is known (minimally 25 days interval) and no new dourine cases have emerged in the herd.

4.3.9. Should euthanasia be systematically applied to infected horses?

The Scientific Committee recommends to systematically apply euthanasia or slaughtering under the conditions put forward in chapter 4.3.8.

4.3.10. Should castration be considered as a control measure?

The Scientific Committee is of the opinion that castration is not a valuable alternative measure as infection can be transmitted by horseflies in the case of *T. evansi*.

4.3.11. Which control measures are recommended for horses having had contact with an infected animal or an animal suspected to be infected? Which kind of contacts should be accounted for?

Transmission of infection between animals can occur via venereal (*T. equiperdum*) and mechanical way (*T. evansi*).

In case of contact of an animal with a confirmed case of dourine the Scientific Committee recommends to apply the following measures:

- to perform two paired serological CFT tests with 25 days interval,
- to put the animal in quarantine during at least 25 days or until the result of the 2nd CFT test is known and no other clinical cases of dourine have emerged in the herd.

In case of animals suspected to be infected because of an epidemiological link with a confirmed case the Scientific Committee refers to the measures described under point 4.3.3.

In case of contact of an animal with a suspected case of dourine the Scientific Committee recommends to put the animal in quarantine while waiting for the results of two paired serological CFT tests with 25 days interval.

If an animal is a suspected case of dourine the Scientific Committee recommends to wait for the results of the 2nd test.

In the literature (Hagos et al., 2010) dexamethasone has been used to induce immunosuppression in experimentally infected horses who developed a chronic (aparasitaemic) form of dourine. Parasitaemia was induced within 7 days after a five-day treatment showing the potential to increase success rates of parasite isolation of *T. equiperdum* from chronic dourine cases or to confirm parasite presence in seropositive yet apparently aparasitaemic cases of dourine.

5. Conclusions

The Scientific Committee is of the opinion that the risk of introduction of dourine in Belgium is very low but that vigilance is certainly warranted given the globalization of trade in semen and intercontinental travelling of horses. The diagnosis of dourine is a challenge and is based on a combination of presence of clinical symptoms, results of serological tests and presence of an epidemiological link with the disease. Given the fact that Belgium is free from dourine it is recommended to slaughter or euthanize a confirmed case as soon as possible to limit the spread of the disease. Castration is not a valuable alternative measure as infection can be transmitted by horseflies.

6. Recommendations

The Scientific Committee recommends to make up adequate legislation in order to have the necessary tools in place in case of introduction of dourine on the territory.

For the Scientific Committee,
The Chairman,

Prof. Dr. E. Thiry (Sgd.)
Brussels, 17/06/2016

References

- AHT / BEVA / DEFRA Equine Quarterly Disease Surveillance Report 2011, Volume 6, Volume 7, 2. Dourine – An emerging venereal threat to European horses.
- Brun R., Hermann H., Zhao-Rong L., 1998. *Trypanosoma evansi* and *Trypanosoma equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Vet. Parasitol.* 79, 95-107.
- Cauchard J., Soldan A., Madeline A., Johnson P., Büscher P., Petry S. 2014. Inter-laboratory ring trials to evaluate serological methods for dourine diagnosis. *Veterinary Parasitology* 205, 70-76.
- Center for Food Security & Public Health (CFSPH) – Iowa State University, 2015. Dourine
- Claes F., Büscher P., Touratier L., Goddeeris B., 2005. *Trypanosoma equiperdum*: master of disguise or historical mistake? *Trends in Parasitology*, 21, 7, 316-320.
- Claes F., Dujardin J.C., Touratier L., Büscher P., Goddeeris B., 2006. Response to Li et al. and Shaw: return of the ring – opportunities to challenge a hypothesis. *Trends in Parasitology*, 22, 2, 58-59.
- Hagos A., Goddeeris B., Yilkal K., Alemu T., Fikru R., Yacob H., Feseha G., Claes F., 2010. Efficacy of Cymelarsan and Diminisan against *Trypanosoma equiperdum* infections in mice and horses. *Veterinary Pathology* 171, 200-206.
- Li F., Lai D., Lukes J., Chen X, Lun Z., 2006. Doubts about *Trypanosoma equiperdum* strains classed as *Trypanosoma brucei* or *Trypanosoma evansi*. *Trends in Parasitology*, 22, 2, 55-56.
- OIE, 2013. Terrestrial Manual. Chapter 2.5.3. Dourine.
- OIE, 20/07/2015. Terrestrial Animal Health Code. Chapter 12.3. Dourine.
- Pascucci I., Di Provido A., Camma C., Di Francesco G., Calistri P., Tittarelli M., Ferri N., Scacchia M., Caporale V., 2013. Diagnosis of dourine outbreaks in Italy. *Vet Parasitol*, 193, 124-133.

Presentation of the Scientific Committee of the FASFC

The Scientific Committee is an advisory body of the Belgian Federal Agency for the Safety of the Food Chain (FASFC) that provides **independent scientific opinions** on risk assessment and risk management in the food chain, and this at the request of the Chief Executive Officer of the FASFC, the Minister competent for food safety or at its own initiative. The Scientific Committee is administratively and scientifically supported by the Staff direction for Risk Assessment of the Agency.

The Scientific Committee consists of 22 members who are appointed by royal decree on the basis of their scientific expertise in areas related to the safety of the food chain. When preparing an opinion, the Scientific Committee can call on external experts who are not a member of the Scientific Committee. Similar to the members of the Scientific Committee, they must be able to work independently and impartially. To ensure the independence of the opinions, potential conflicts of interest are managed transparently.

The opinions are based on a scientific assessment of the question. They express the view of the Scientific Committee which is taken in consensus on the basis of a risk assessment and the existing knowledge on the subject.

The opinions of the Scientific Committee may contain **recommendations** for food chain control policy or for the stakeholders. The follow-up of these recommendations for control policy is the responsibility of the risk managers.

Questions about an opinion can be directed to the secretariat of the Scientific Committee:

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Members of the Scientific Committee

The Scientific Committee is composed of the following members:

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Conflict of interest

Because of a potential conflict of interest T. van den Berg (CODA-CERVA), P. Büscher (ITG) and D. Fretin (CODA-CERVA), involved in the activities of the dourine reference laboratory, participated as 'heard experts' in the workgroup.

T. van den Berg, member of the SciCom, did not participate in the deliberations in regard to the opinion during the plenary session of the Scientific Committee.

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Composition of the workgroup

The workgroup was composed of:

Members of the Scientific Committee: D. Berkvens (reporter)

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File manager: X. Van Huffel (FASFC)

The activities of the workgroup were attended by the following members of the administration (as observers): C. Rettigner (FASFC) and D. Tamignaux (FPS Public Health, Food Safety and Environment).

Legal framework

Law of 4 February 2000, on the creation of the Federal Agency for the Safety of the Food Chain, in particular article 8;

The Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established within the Federal Agency for the Safety of the Food Chain;

The Internal Rules as mentioned in Article 3 of the Royal Decree of 19 May 2000, on the composition and operating procedures of the Scientific Committee, as established within the Federal Agency for the Safety of the Food Chain, approved by the Minister on 9 June 2011.

Disclaimer

The Scientific Committee at all times reserves the right to modify the opinion by mutual consent, should new information and data become available after the publication of this version.