SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 14 September 2017

doi: 10.2903/j.efsa.2017.4992

Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): Trichomonosis

EFSA Panel on Animal Health and Welfare (AHAW),
Simon More, Anette Bøtner, Andrew Butterworth, Paolo Calistri, Klaus Depner, Sandra Edwards, Bruno Garin-Bastuji, Margaret Good, Christian Gortázar Schmidt, Virginie Michel, Miguel Angel Miranda, Søren Saxmose Nielsen, Mohan Raj, Liisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Antonio Velarde, Preben Willeberg, Christoph Winckler, Francesca Baldinelli, Alessandro Broglia, Denise Candiani, Beatriz Beltrán-Beck, Lisa Kohnle and Dominique Bicout

Abstract

Trichomonosis has been assessed according to the criteria of the Animal Health Law (AHL), in particular criteria of Article 7 on disease profile and impacts, Article 5 on the eligibility of trichomonosis to be listed, Article 9 for the categorisation of trichomonosis according to disease prevention and control rules as in Annex IV and Article 8 on the list of animal species related to trichomonosis. The assessment has been performed following a methodology composed of information collection and compilation, expert judgement on each criterion at individual and, if no consensus was reached before, also at collective level. The output is composed of the categorical answer, and for the questions where no consensus was reached, the different supporting views are reported. Details on the methodology used for this assessment are explained in a separate opinion. According to the assessment performed, trichomonosis can be considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL. The disease would comply with the criteria as in sections 3, 4 and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (c), (d) and (e) of Article 9(1). The animal species to be listed for trichomonosis according to Article 8(3) criteria is cattle as susceptible and reservoir.

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Trichomonosis, tritrichomonosis, *Tritrichomonas foetus*, Animal Health Law, listing, categorisation, impact

Requestor: European Commission

Question number: EFSA-Q-2017-00146

Correspondence: alpha@efsa.europa.eu

Panel members: Dominique Bicout, Anette Bøtner, Andrew Butterworth, Paolo Calistri, Klaus Depner, Sandra Edwards, Bruno Garin-Bastuji, Margaret Good, Christian Gortázar Schmidt, Virginie Michel, Miguel Angel Miranda, Simon More, Søren Saxmose Nielsen, Mohan Raj, Liisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Antonio Velarde, Preben Willeberg and Christoph Winckler.

Acknowledgements: The Panel wishes to thank Luis Miguel Ortega Mora, Esther Collantes-Fernández and Gereon Schares for the support provided to this scientific output.

Suggested citation: EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More S, Bøtner A, Butterworth A, Calistri P, Depner K, Edwards S, Garin-Bastuji B, Good M, Gortázar Schmidt C, Michel V, Miranda MA, Nielsen SS, Raj M, Sihvonen L, Spoolder H, Stegeman JA, Thulke H-H, Velarde A, Willeberg P, Winckler C, Baldinelli F, Broglia A, Candiani D, Beltrán-Beck B, Kohnle L and Bicout D, 2017. Scientific Opinion on the assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): Trichomonosis. EFSA Journal 2017;15(10):4992, 37 pp. https://doi.org/10.2903/j.efsa.2017.4992

ISSN: 1831-4732

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the Creative Commons Attribution-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

Reproduction of the images listed below is prohibited and permission must be sought directly from the copyright holder:

Figures 1 and 2 (Annex): © World Organization for Animal Health (OIE); Table 3 and Table 3 (Annex): © World Organization for Animal Health (OIE)

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

Abstract ... 1
1. Introduction ... 4
1.1. Background and Terms of Reference as provided by the requestor ... 4
1.2. Interpretation of the Terms of Reference ... 4
2. Data and methodologies ... 4
3. Assessment ... 4
3.1. Assessment according to Article 7 criteria ... 4
3.1.1. Article 7(a) Disease Profile ... 4
3.1.1.1. Article 7(a)(i) Animal species concerned by the disease ... 4
3.1.1.2. Article 7(a)(ii) The morbidity and mortality rates of the disease in animal populations ... 6
3.1.1.3. Article 7(a)(iii) The zoonotic character of the disease ... 7
3.1.1.4. Article 7(a)(iv) The resistance to treatments, including antimicrobial resistance ... 7
3.1.1.5. Article 7(a)(v) The persistence of the disease in an animal population or the environment ... 7
3.1.1.6. Article 7(a)(vi) The routes and speed of transmission of the disease between animals, and, when relevant, between animals and humans ... 8
3.1.1.7. Article 7(a)(vii) The absence or presence and distribution of the disease in the Union, where the disease is not present in the Union, the risk of its introduction into the Union ... 9
3.1.1.8. Article 7(a)(viii) The existence of diagnostic and disease control tools ... 11
3.1.2. Article 7(b) The impact of diseases ... 13
3.1.2.1. Article 7(b)(i) The impact of the disease on agricultural and aquaculture production and other parts of the economy ... 13
3.1.2.2. Article 7(b)(ii) The impact of the disease on human health ... 14
3.1.2.3. Article 7(b)(iii) The impact of the disease on animal welfare ... 14
3.1.2.4. Article 7(b)(iv) The impact of the disease on biodiversity and the environment ... 15
3.1.3. Article 7(c) Its potential to generate a crisis situation and its potential use in bioterrorism ... 15
3.1.4. Article 7(d) The feasibility, availability and effectiveness of the following disease prevention and control measures ... 15
3.1.4.1. Article 7(d)(i) Diagnostic tools and capacities ... 15
3.1.4.2. Article 7(d)(ii) Vaccination ... 16
3.1.4.3. Article 7(d)(iii) Medical treatments ... 17
3.1.4.4. Article 7(d)(iv) Biosecurity measures ... 18
3.1.4.5. Article 7(d)(v) Restrictions on the movement of animals and products ... 18
3.1.4.6. Article 7(d)(vi) Killing of animals ... 19
3.1.4.7. Article 7(d)(vii) Disposal of carcasses and other relevant animal by-products ... 19
3.1.5. Article 7(e) The impact of disease prevention and control measures ... 20
3.1.5.1. Article 7(e)(i) The direct and indirect costs for the affected sectors and the economy as a whole ... 20
3.1.5.2. Article 7(e)(ii) The societal acceptance of disease prevention and control measures ... 20
3.1.5.3. Article 7(e)(iii) The welfare of affected subpopulations of kept and wild animals ... 21
3.1.5.4. Article 7(e)(iv) The environment and biodiversity ... 21
3.2. Assessment according to Article 5 criteria ... 21
3.2.1. Outcome of the assessment of trichomonosis according to criteria of Article 5(3) of the AHL on its eligibility to be listed ... 22
3.3. Assessment according to Article 9 criteria ... 22
3.3.1. Non-consensus-questions ... 24
3.3.2. Outcome of the assessment of criteria in Annex IV for trichomonosis for the purpose of categorisation as in Article 9 of the AHL ... 25
3.4. Assessment of Article 8 ... 26
4. Conclusions ... 27
References ... 27
Abbreviations ... 33
Appendix A – Diagnosis of trichomonosis ... 34
Annex A – Mapped fact-sheet used in the individual judgement on trichomonosis ... 37

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The background and Terms of Reference (ToR) as provided by the European Commission for the present document are reported in Section 1.2 of the scientific opinion on the ad hoc methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9 and 8 within the Animal Health Law (AHL) framework (EFSA AHAW Panel, 2017).

1.2. Interpretation of the Terms of Reference

The interpretation of the ToR is as in Section 1.2 of the scientific opinion on the ad hoc methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9 and 8 within the AHL framework (EFSA AHAW Panel, 2017).

The present document reports the results of assessment on trichomonosis according to the criteria of the AHL articles as follows:

- Article 7: trichomonosis profile and impacts
- Article 5: eligibility of trichomonosis to be listed
- Article 9: categorisation of trichomonosis according to disease prevention and control rules as in Annex IV
- Article 8: list of animal species related to trichomonosis.

2. Data and methodologies

The methodology applied in this opinion is described in detail in a dedicated document about the ad hoc method developed for assessing any animal disease for the listing and categorisation of diseases within the AHL framework (EFSA AHAW Panel, 2017).

3. Assessment

3.1. Assessment according to Article 7 criteria

This section presents the assessment of trichomonosis according to the Article 7 criteria of the AHL and related parameters (see Table 2 of the opinion on methodology (EFSA AHAW Panel, 2017)), based on the information contained in the fact-sheet as drafted by the selected disease scientist (see section 2.1 of the scientific opinion on the ad hoc methodology) and amended by the AHAW Panel.

3.1.1. Article 7(a) Disease Profile

3.1.1.1. Article 7(a)(i) Animal species concerned by the disease

Susceptible animal species

Parameter 1 – Naturally susceptible wildlife species (or family/orders)

Not applicable – *Tritrichomonas foetus* has not been reported so far in wildlife species. An early report in roe deer (*Capreolus capreolus*) has not been confirmed.

Parameter 2 – Naturally susceptible domestic species (or family/orders)

Natural hosts of bovine *T. foetus* are cattle (*Bos taurus*) and zebu (*Bos taurus indicus*). The parasite is located in the genital tract of cattle (Skirrow and BonDurant, 1988; BonDurant, 2005; Sager et al., 2007) causing trichomonosis (syn. tritrichomonosis).

Despite *T. foetus* or similar (i.e. *Tritrichomonas suis*) species found infecting cats and pigs, evidences from genetic and biology point of view suggest that there are no links between life cycles of *T. foetus* in cattle and cats or between life cycles of feline or bovine *T. foetus* and porcine *T. suis*. Most likely these parasites have evolved separately and despite their similarity, *T. foetus* of bovine and feline origin as well as porcine *T. suis* show biological traits which differ considerably. Therefore, each form is regarded as genetically distinct (Slapeta et al., 2010, 2012). More detailed information is provided below.

T. foetus has been confirmed as the causative agent of chronic diarrhoea in the domestic cat (*Felis catus*) (Gookin et al., 1999; Levy et al., 2003). There are also biological differences between isolates from cattle and cats concerning pH tolerance (Morin-Adeline et al., 2015); route of transmission (venereal versus faecal–oral), pathogenicity in experimental infections of uterus of cows (Stockdale et al., 2007) and survival in invertebrate vectors (slugs) (Van der Saag et al., 2011). Thus, some authors believe that feline *T. foetus* represents a different species and a change in name, to *Tritrichomonas blagburni*, has been proposed (Walden et al., 2013).

Trichomonads are occasionally observed in the faeces from dogs (*Canis familiaris*) with diarrhoea (Gookin et al., 2005). The parasite has also been detected in the faeces of puppies, indicating that *T. foetus* may parasitise dogs (Grellet et al., 2010). Finally, *T. foetus* isolated from cattle seems to be morphologically and genetically identical to *Trichomonas suis*, that is a commensal observed in the nasal cavity, stomach, caecum and colon of the domestic pig (*Sus scrofa domesticus*) (Felleisen et al., 1998; Hampl et al., 2001; Tachezy et al., 2002; Reinmann et al., 2012; Slapeta et al., 2012; Sun et al., 2012). Consequently, it was assumed that *T. suis* and *T. foetus* belong to the same species (Tachezy et al., 2002; Lun et al., 2005; Frey and Müller, 2012; Yao and Köster, 2015). However, more recent epidemiological studies suggest that cross-species transmission from pigs to cattle on the same farm (e.g. by exposure to *T. foetus*-contaminated pig faeces) is unlikely to occur (Mueller et al., 2015).

Parameter 3 – Experimentally susceptible wildlife species (or family/orders)

Not applicable – *T. foetus* has not been reported so far in wildlife species.

Parameter 4 – Experimentally susceptible domestic species (or family/orders) (Table 1)

Table 1: Domestic species susceptible to experimental *T. foetus* infection

Animal species		Inoculation route	Infection/clinical signs	Reference
Bos taurus	Bulls	Preputium	Yes/asymptomatic and/or mild lesions in preputial mucosa	Clark et al. (1974)
	Heifers (non-pregnant)	Vagina	Yes/vaginitis, cervicitis and endometritis	Parsonson et al. (1976), Skirrow and BonDurant (1990), BonDurant et al. (1993), Anderson et al. (1996)
Felis catus		Orogastric	Yes/chronic or intermittent large bowel diarrhoea	Gookin et al. (2001)

Intravaginal *T. foetus* infection has been achieved in different laboratory animal models such as golden hamster (Ryley and Stacey, 1963), mice with (St Claire et al., 1994) or without (Mutwiri and Corbeil, 1998) oestrogen treatment prior to inoculation and in pregnant mice (Agnew et al., 2008; Barbeito et al., 2008).

Reservoir animal species

Parameter 5 – Wild reservoir species (or family/orders)

Not applicable – confirmed cases of *T. foetus* have not been described so far in wildlife species.

Parameter 6 – Domestic reservoir species (or family/orders)

Infection in bulls is reported to persist for more than 3 years and may persist for life (Flower et al., 1983; Campero et al., 1990; Rhyan et al., 1999).

Carrier cows may represent a reservoir of infection for bulls. A very small proportion of cows (a fraction less than 1%) in infected herds have been shown to remain infected throughout pregnancy, and into the following breeding season (BonDurant, 2005). Carrier dams have been reported; e.g. two chronically infected dams were observed in one Australian study 16 and 22 months after initial infection (Alexander, 1953). In a Californian dairy herd, infected cows were found positive 9 weeks after pregnancy (Skirrow, 1987) or 63 days after parturition (Goodger and Skirrow, 1986). In a more recent study from Argentina, several heifers remained infected up to 300 days after breeding (Mancebo et al., 1995).

3.1.1.2. Article 7(a)(ii) The morbidity and mortality rates of the disease in animal populations

Morbidity

Parameter 1 – Prevalence/Incidence

Trichomonosis in bovine is a major problem worldwide, mainly in beef herds managed under extensive conditions and where natural service is used. The infection has been described in all the continents where cattle are raised, but the percentage of infected animals or herds varies widely between regions. Prevalence is usually lower in areas where control programmes exist (Table 2).

Table 2: Infection rates (agent isolation) of *T. foetus* in cattle throughout the world

Country	Region	Type of sample	% Herds positive	% Animals positive	Control programme	References
Argentina	Buenos Aires	Preputial sample	24	NA	No	Mardones et al. (2008)
	La Pampa	Preputial sample	5.1	1.1	Yes	Molina et al. (2013)
Australia	Victoria River district	Preputial sample	65.5	8	No	McCool et al. (1988)
Brazil	Pernambuco	Cervico-vaginal mucus sample	90.5	33.4	No	Oliveira et al. (2015)
China	Beijing	Abomasal content of aborted fetuses	NA	5	No	Yang et al. (2012)
Costa Rica	NA	Preputial sample	18.4	7.2	No	Pérez et al. (1992)
South Africa	North Western Cape	Preputial sample	NA	10.2	No	Erasmus et al. (1989)
Spain	Asturias	Preputial sample	41.5	32	No	Mendoza-Ibarra et al. (2012)
		Preputial sample	18.7–19.7	12.7–13.6	Yes	Collantes-Fernández et al. (2014)
Turkey	Anatolia	Abomasal content of aborted fetuses	NA	5.7	No	Guven et al. (2013)
USA	Texas	Preputial sample	NA	3.7	Yes	Szonyi et al. (2012)
	California	Preputial sample	15.8	4.1	No	BonDurant et al. (1990)
	Idaho	Preputial sample	40.9	NA	No	Gay et al. (1996)
	Florida	Preputial sample	30.4	6	No	Rae et al. (2004)
	Wyoming	Preputial sample	NA	0.2	Yes	Yao et al. (2011)
		Cervico-vaginal mucus sample	NA	9.7	Yes	Yao (2015)
	Alabama	Preputial sample	NA	0–0.27	No	Rodning et al. (2008)

In European Union (EU) countries, the disease presence has dramatically decreased in recent years or even has been eradicated (in dairy cattle) due to the implementation of control programmes and the use of artificial insemination (AI). A recent survey conducted in Switzerland involving 1,362 preputial samples from bulls and 60 abomasal fluid samples of aborted fetuses from beef and dairy herds revealed no *T. foetus* positive finding (Bernasconi et al., 2014). However, other studies suggest a re-emergence of the disease in extensive production systems in southern Europe. In Spain, 32% of the tested bulls were infected, positive at culture or by polymerase chain reaction (PCR) preputial samples (Mendoza-Ibarra et al., 2012). It is to be noted that, since *T. foetus* infection is asymptomatic in bulls, prevalence (percentage of bulls with a preputial sample positive by culture or PCR) is not linked to morbidity in bulls. However, there is a positive association between bull infection rates and the proportion of non-pregnant cows and heifers in a herd. The presence of trichomonosis was reported to World Organization for Animal Health (OIE) by several countries in Europe (Table 3).

Parameter 2 – Case-morbidity rate (% clinically diseased animals out of infected ones)

In males, after inoculation of *T. foetus* in 3–7-year old bulls, 92.3% of animals were infected (Clark et al., 1974). However, *T. foetus* infection is asymptomatic in bulls and affects neither semen quality nor sexual behaviour (Parsonson et al., 1974; Rhyan et al., 1999), thus morbidity rate is 0% for bulls. However, there is a positive association between bull infection rates and the proportion of non-pregnant cows and heifers in a herd.

Few data about percentage of clinically diseased cows in Europe are reported in the literature. In infected herds, low overall pregnancy rate is noted and in newly exposed herds it can be as low as 50% of the expected rate (BonDurant, 2005). Distribution of cases in an infected herd was reported in 8% aborted cows, 2% pyometra, 60% infertility (late breeders), 20% infected cows without any losses and 10% non-infected (NDA, online).

Mortality

Parameter 3 – Case-fatality rate

Infection causes no adult mortality; neither in bulls nor cows is fatal cases reported. Infection of pregnant cattle can result in early fetal death. Abortions of fetuses typically occur about 2 months gestational age (at > 42 days of gestation with a peak loss at 70–90 days) (Parsonson et al., 1976; BonDurant, 2005). Fifty per cent of pregnant exposed cows which resulted positive subsequently aborted (Kimsey, 1986).

3.1.1.3. Article 7(a)(iii) The zoonotic character of the disease

Only four reports of zoonotic cases are available in literature and they are the only cases reported worldwide so far. In such cases, *T. foetus*-like organisms have been reported as opportunistic infections in immunocompromised or immunosuppressed individuals (Okamoto et al., 1998; Duboucher et al., 2006; Zalonis et al., 2011; Suzuki et al., 2016). Data on human cases have to be interpreted with care as only for the most recent case described, was enough molecular data available to suspect infection with *T. foetus*. It is suspected that some of those cases were caused by the *T. foetus* cattle/swine genotype based on molecular characterisation. The symptoms and location of lesions differed largely in the human cases reported; *T. foetus*-like tritrichomonads have been detected in cerebrospinal fluid (meningoencephalitis) (Okamoto et al., 1998), broncheoalveolar lavage specimens (pneumonia) (Duboucher et al., 2006), peritoneal fluid (peritonitis) (Zalonis et al., 2011) and biliary tract (Suzuki et al., 2016).

3.1.1.4. Article 7(a)(iv) The resistance to treatments, including antimicrobial resistance

Parameter 1 – Resistant strain to any treatment even at laboratory level

In the past, various imidazole compounds (metronidazole, dimetridazole and ipronidazole) were used to treat *T. foetus*-infected bulls, and drug resistant strains to these imidazole compounds were found (BonDurant, 2005; Rae and Crews, 2006). Currently, there is no approved treatment for cattle infected with *T. foetus* because of concerns regarding toxic residues in meat (BonDurant, 1997).

3.1.1.5. Article 7(a)(v) The persistence of the disease in an animal population or the environment

Animal population

Parameter 1 – Duration of infectious period in animals

The infected bull acts as an asymptomatic carrier throughout his life (Clark et al., 1974; Parsonson et al., 1974; Parker et al., 1999).

In the female, 2 weeks after infection, *T. foetus* may have colonised the different parts of the genital tract (Parsonson et al., 1976). In the female, the infection is self-limiting and most cows are able to clear the infection after a few months, usually after 1–3 months, rarely longer (Parsonson et al., 1976; Skirrow and BonDurant, 1990; BonDurant, 2005), which can be considered the normal duration of the infectious period in females (see Section 3.1.1.1 Parameter 5 and Parameter 3 below for reported exceptions to this normality). The preferred location is in the cervix and cervico-vaginal mucus, but the number of parasites varies throughout the oestrus cycle, being higher in the days prior to oestrus. The establishment of the parasite in the genital tract of the female does not seem to interfere with the fertilisation nor with the early development of the embryo (Bielanski et al., 2004). In heifers, experimentally infected with *T. foetus*, conceptus deaths peaked at 50–70 days of gestation

(Parsonson et al., 1976). Occasional abortions of fetuses older than 4 month gestational age are reported, but typically losses occurs 2 months earlier (BonDurant, 2005).

Parameter 2 – Presence and duration of latent infection period

Bulls remain persistently infected for more than 3 years and infection may persist for life (Flower et al., 1983; Campero et al., 1990; Rhyan et al., 1999; BonDurant, 2005).

The infection in the female is usually self-limiting, disappearing between 2 and 4 months after the loss of the conceptus with some reported exceptions. The immunity that develops is not permanent and usually lasts for about 6 months; after 6 months, the female is again susceptible to infection (Parsonson et al., 1976; BonDurant, 2005).

Parameter 3 – Presence and duration of the pathogen in healthy carriers

Infection in bulls is asymptomatic and persists for more than 3 years and probably for life (Flower et al., 1983; Campero et al., 1990; Rhyan et al., 1999). Bulls younger than 4 years are less often carriers of *T. foetus* (Kimsey et al., 1980; BonDurant, 1985; Skirrow et al., 1985; BonDurant et al., 1990; Pérez et al., 1992; Ondrak, 2016).

Carrier dams have been reported; not all cows are able to clear infection prior to re-breeding and some remain infected for up to almost 1 year, even after parturition (Alexander, 1953; Goodger and Skirrow, 1986; Skirrow, 1987; Mancebo et al., 1995). These cows represent carrier cows, e.g. two chronically infected dams were observed in one Australian study 16 and 22 months after initial infection (Alexander, 1953). In a Californian dairy herd, infected cows were found positive 9 weeks after pregnancy (Skirrow, 1987) or 63 days after parturition (Goodger and Skirrow, 1986). In a more recent study from Argentina, several heifers remained infected up to 300 days after breeding (Mancebo et al., 1995) which underlines the importance of carrier state heifers for persistence of infection in affected herds.

Environment

Parameter 4 – Length of survival (dpi) of the agent and/or detection of DNA in selected matrices (soil, water, air) from the environment (scenarios: high and low T)

Bovine *T. foetus* is considered incapable of forming cysts and viability of the parasite in the environment is very limited (BonDurant and Honigberg, 1994).

3.1.1.6. Article 7(a)(vi) The routes and speed of transmission of the disease between animals, and, when relevant, between animals and humans

Routes of transmission

Parameter 1 – Types of routes of transmission from animal to animal (horizontal, vertical)

Trichomonosis in bovine is horizontally transmitted and vertical transmission plays no role in the spread of the disease. Transmission of the infection during pregnancy to the embryo or the foetus may result in embryonic death, resorption or abortion.

Trichomonosis is a venereal disease transmitted from an infected bull to an uninfected dam or vice versa (Ondrak, 2016) almost exclusively during coitus. In general, a transmission rate of 30–70% between infected bulls and female cattle is assumed (BonDurant, 2005); however, a single mating with an infected bull may result in an up to 95% infection rate among susceptible heifers (Parsonson et al., 1976).

Mechanical transmission, either by uninfected bulls (Clark et al., 1977; Ondrak, 2016) or iatrogenic, e.g. using the same glass rod or insemination pipette for different cows (Murname, 1959), not properly disinfected specula (Goodger and Skirrow, 1986) or contaminated cryopreserved semen (Blackshaw and Beattie, 1955; Clark et al., 1971; Skirrow and BonDurant, 1988) seems to be possible but the frequency of these events is very rare. Mechanical transmission seems to be possible through a healthy bull, i.e. from an infected cow to a receptive cow, if there is not too much time between two services (less than 20 minutes) (Clark et al., 1977; Goodger and Skirrow, 1986; Ondrak, 2016).

There is no evidence to support the persistence of *T. foetus* in the environment and its further transmission to susceptible animals.

Parameter 2 – Types of routes of transmission between animals and humans (direct, indirect, including food-borne)

To date, only few cases of human trichomonosis caused by *T. foetus* of the cattle/swine genotype have been reported, as rare opportunistic infections in immunosuppressed or immunologically impaired

individuals (Okamoto et al., 1998; Duboucher et al., 2006; Zalonis et al., 2011; Suzuki et al., 2016). Routes of infection are unknown and it was suspected that individuals with intestinal damage due to pre- or co-infection with another agent are susceptible after oral infection (Suzuki et al., 2016).

Speed of transmission

Parameter 3 – Incidence between animals and, when relevant, between animals and humans

There are no figures on incidence in literature.

Parameter 4 – Transmission rate (beta) (from R_0 and infectious period) between animals and, when relevant, between animals and humans

A precise transmission rate has not yet been determined. There is no literature available reporting on 'beta'. Statistical modelling was performed with assumptions regarding transmission rates based on literature data (Villarroel et al., 2004). When a bovine herd did not test bulls for trichomonosis before the breeding season, a triangular probability distribution for both, young bulls (minimum, 0%; mode, 5%; maximum, 40%) and old bulls (minimum, 0%; mode, 35%; and maximum, 80%) to transmit an existing infection to non-infected dams was assumed. For a transmission from infected cows to susceptible bulls, a triangular distribution of probabilities (minimum, 0%; mode, 0%; maximum, 50%) was assumed for young bulls and a uniform probability distribution for old bulls (minimum, 50%; maximum, 100%) (Villarroel et al., 2004).

3.1.1.7. Article 7(a)(vii) The absence or presence and distribution of the disease in the Union, where the disease is not present in the Union, the risk of its introduction into the Union

Presence and distribution

Trichomonosis has been reported to the OIE in the last 5 years in countries from the EU such as Spain, Portugal, France, Hungary and Poland (Table 3).

Table 3: Trichomonosis status in Countries in Europe based on World Animal Health Information Database (WAHIS Interface) from 2012 to 2016

EU country	2012	2013	2014	2015	2016
Albania	No information available	No information available	Infection limited to one or more zones	Disease present	Infection
Austria	No information available	No information available	No information available	No information available	No information available
Belgium	Disease absent	Infection	Disease absent	Disease absent	Disease absent
Bosnia and Herzegovina	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Bulgaria	No information available	No information available	Disease absent	Disease absent	Disease absent
Croatia	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Cyprus	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Czech Republic	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Denmark	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Estonia	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Finland	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
France	Disease present	Disease present	Disease present	Disease present	Disease present
Germany	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Greece	Disease absent	Disease absent	Disease absent	Disease absent	No information available
Hungary	Disease limited to one or more zones	Disease limited to one or more zones	Disease limited to one or more zones	Disease limited to one or more zones	Disease absent
Ireland	No information available	No information available	No information available	No information available	No information available

EU country	2012	2013	2014	2015	2016
Italy	Disease absent	Infection/ infestation limited to one or more zones	Disease absent	Infection/ infestation limited to one or more zones	Disease absent
Latvia	Never reported	Never reported	Never reported	Never reported	Never reported
Lithuania	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Luxembourg	Disease absent	Disease absent	Disease absent	Disease absent	No information available
Malta	Never reported	Never reported	Never reported	Never reported	Never reported
Montenegro	Disease absent	Disease absent	Disease absent	No information available	No information available
Netherlands	Infection	Infection	Infection	Infection	Infection
Norway	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Poland	Infection	Disease absent	Disease absent	Disease absent	Disease absent
Portugal	Disease absent	Disease absent	Disease absent	Disease present	Disease present
Romania	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Serbia	Disease absent	Disease absent	Disease absent	Disease absent	No information available
Slovakia	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
Slovenia	Never reported	Never reported	Never reported	Never reported	Never reported
Spain	Disease limited to one or more zones	Disease limited to one or more zones	Disease limited to one or more zones	Disease limited to one or more zones	Disease limited to one or more zones
Sweden	Never reported	Never reported	Never reported	Never reported	Never reported
Switzerland	Disease absent	Disease absent	Disease absent	Disease absent	Disease absent
United Kingdom	Disease present	Disease present	Disease present	Disease present	Disease present

Parameter 2 – Type of epidemiological occurrence (sporadic, epidemic, endemic) at MS level

In the EU, the use of AI and effective control programmes have greatly reduced the incidence of trichomonosis in bovine and the disease is largely eradicated in dairy cattle herds. The infection is limited to beef cattle making use of natural mating. No information is available about the epidemiological occurrence of the infection in European countries where the disease has been reported to the OIE (i.e. France, Portugal, Hungary and Poland, see Table 3), except for some regions of Spain where the disease is endemic (Mendoza-Ibarra et al., 2012, 2013).

Risk of introduction

Parameter 3 – Routes of possible introduction

The disease can be introduced by the trade in animals, both males and females with unknown health status. In the EU, there are no global mandatory regulations for cattle movements with regard to trichomonosis in bovine. Recommendations for the importation of cattle and bulls for breeding can be found in the OIE Terrestrial Animal Health Code. In particular, this includes testing bulls and allowing the importation of *T. foetus*-free bulls only for reproductive purposes. Positive bulls need to be culled (Yao, 2013).

The disease can be also introduced by semen trade used for AI due to the fact that *T. foetus* may be present in semen if it is contaminated with preputial fluid during manual collection (BonDurant, 2005) and the parasite can survive the freezing process used to preserve semen. In the EU, specific regulations are applied to bovine semen trade and to administer the sanitary conditions required for collection centres and the animals (see Section 3.1.4.5).

Parameter 4 – Number of animal moving and/or shipment size

Numbers of live cattle for breeding purpose and bovine semen trade between EU countries and from third countries into the EU can be consulted in TRACES (TRACES, online).

Parameter 5 – Duration of infectious period in animal and/or commodity

Bulls can shed the parasite indefinitely (Parsonson et al., 1974; Rhyan et al., 1999).

In the female, the infection is self-limiting and, with some exceptions, the parasite normally disappears simultaneously from all areas of the genital tract after a period of at least 90–95 days (Parsonson et al., 1976; Rae et al., 2004; BonDurant, 2005). A low percentage (about 1%) of the dams remains infected until the next breeding period, and so is still able to infect bulls.

Parameter 6 – List of control measures at border (testing, quarantine, etc.)

There are no global mandatory regulations for cattle movement with regard to trichomonosis.

Parameter 7 – Presence and duration of latent infection and/or carrier status

The infected bulls remain asymptomatic carriers representing a permanent source of infection. A very small proportion of cows (a fraction less than 1%) in infected herds have been shown to remain infected throughout pregnancy, and in the following breeding season, also representing carrier animals (BonDurant, 2005).

Parameter 8 – Risk of introduction

The disease is already present in the EU.

3.1.1.8. Article 7(a)(viii) The existence of diagnostic and disease control tools

Diagnostic tools

Parameter 1 – Existence of diagnostic tools

Optimal for the diagnosis of trichomonosis is the diagnostic cultivation of the sampled material followed by daily microscopic examination of the culture to observe and characterise multiplying trichomonads. Several media have been used successfully (OIE, 2017a). Transport and cultivation kits are commercially available (e.g. InPouch TF, BioMed Diagnostics, White City, OR, USA). The identity of cultivated trichomonads can be confirmed after fixation and staining (Lun and Gajadhar, 1999) or optimally by PCR to avoid false positives (Parker et al., 2001; Campero et al., 2003).

Alternatively, sampled material can be analysed for parasitic DNA without prior cultivation. A large number of PCRs have been reported. The majority of diagnostic PCRs published are targeting the DNA coding for ribosomal ribonucleic acid (rRNA) and flanking regions (recombinant DNA (rDNA)). This rDNA region includes the 18S rDNA (small subunit rDNA (SSU rDNA)), the internal transcribed spacer (ITS) 1 region, the 5.8S rDNA, the ITS2 and the 28S rDNA (large subunit rDNA (LSU rDNA)).

Both diagnostic conventional end-point PCRs and diagnostic real-time PCRs (e.g. SYBR green-based, 5′ nuclease assays) have been developed for the detection of trichomonads (Table A.1 in Appendix A). Because rDNA shows only limited differences between trichomonads (Felleisen, 1997), end-point assays have been applied using primer pairs capable to amplify DNA of several trichomonad species simultaneously. In these PCRs, species diagnosis was achieved in a second step, either by PCR restriction fragment length polymorphism (RFLP) (Hayes et al., 2003), by determining the precise size of amplification products (Grahn et al., 2005; Frey et al., 2009) or by single-strand conformation polymorphism (SSCP) (Huby-Chilton et al., 2009). One of the first diagnostic end-point PCRs established and targeting rDNA, i.e. the TFR3/TRF4 PCR (Huby-Chilton et al., 2009) was modified by several groups. A single-tube nested PCR was established using the TFR3/TRF4 primer pair for the external amplification (Gookin et al., 2002). The TFR3/TRF4 PCR was also modified as a SYBR®-based real-time PCR (Mueller et al., 2015). A 5′-nuclease assay (i.e. a real-time PCR applying a Taqman probe) based on rDNA sequences has been established to detect *T. foetus*, *T. suis* and *Tritrichomonas mobilensis* (McMillen and Lew, 2006). In this assay, a 57 bp region of the 5.8S region is amplified. In addition, a commercialised 5′-nuclease assay has been developed (VetMAX™-Gold Trich Detection Kit, Life Technologies) (Effinger et al., 2014), which has been used in epidemiological studies on *T. foetus* from cattle in southern Africa (Casteriano et al., 2016).

Direct microscopic examination without prior cultivation is possible but not recommended due to low sensitivity. Attempts to detect specific antibodies against the parasite have been reported; however, due to both sensitivity and specificity problems, serological tests are not used for routine testing. Of minor importance are antigen detection and DNA hybridisation techniques which have been applied as research tools mainly.

Control tools

Parameter 2 – Existence of control tools

Prevention and control measures are based on the distinctive epidemiologic features of trichomonosis in bovine, a sexually transmitted disease where infected bulls are asymptomatic carriers and represent a permanent source of infection while in heifers and cows infection is typically transient (Clark et al., 1971, 1974; Parsonson et al., 1974; Skirrow and BonDurant, 1990).

In the EU, specific regulations are applied to bovine semen trade and to regulate the sanitary conditions of the collection centre and the animals (Council Directive 88/407/EEC[1] as amended by Council Directive 2003/43/EC[2]). Each Member State ensures that the semen sent from its territory to another Member State is collected from bovine admitted and kept at an approved semen collection centre that has been subjected to a period of quarantine (at least 28 days) and tested for *T. foetus* initially according to their age and afterward once a year (Council Directive 2003/43/EC). Third countries from which Members States can authorise imports into the Union of semen of the bovine species are listed in the Commission Implementing Decision 2011/630/EU[3] laying down animal health conditions and certification requirements for the imports of bovine semen into the Union.

T. foetus may be present in semen if it became contaminated with preputial fluid during manual collection (BonDurant, 2005); recommendations for the importation of cattle and bulls for breeding can be found in the Terrestrial Animal Health Code. In particular, emphasis has been placed in the measures applied to bull semen donor health status to avoid dissemination and transmission of the disease (OIE, 2017b).

In dairy herds and in some beef herds, AI is a very useful measure to reduce and eliminate infection. For bulls destined for AI, quarantine and periodic testing are required. It is of great value to know the country of origin, the reproductive history of the bull, and the tests performed by the artificial insemination centre (Eaglesome and Garcia, 1997).

In herds where it is not possible to introduce AI and natural mating is the normal practice, as in many areas where beef cattle are raised extensively, the following measures are recommended:

Measures to prevent the entrance of disease in trichomonosis free-herds

- Quarantine and testing replacement bulls: Replacement should be done by virgin animals or bulls acquired only from disease-free herds with records of excellence in reproductive performance (Campero and Gottstein, 2007; Yao, 2013; Ondrak, 2016). All the bulls must be tested during quarantine before entrance in the herd. If the bull comes from a trichomonosis-free herd, the analysis of two samples of preputial smegma with 1–2 weeks interval during the quarantine is recommended. Three or more samples must be analysed if bulls are provided from an area where trichomonosis is known to be endemic.
- Avoid communal grazing (mixing animals form different herds) and keep fences in good conditions: These measures will help to control the primary route of transmission (Gay et al., 1996; Mardones et al., 2008; Jin et al., 2014).
- Do not introduce cows or heifers of unknown health status during the breeding season. Similar to bulls, heifers and cows must be acquired only from disease-free herds with records of excellence in reproductive performance.

Measures to control and eradicate trichomonosis in infected herds

- Analysis of bulls before the breeding season and culling of infected bulls: It is recommended to sample the animal two or three times before the breeding season and every time new bulls are introduced into the herds (BonDurant, 2005; Campero and Gottstein, 2007; Yao, 2013).
- Reduce the average age of bulls and replace all bulls after four breeding seasons: Replacement with negative-tested young bulls helps to reduce the prevalence (Clark et al., 1971; Christensen et al., 1977) since 3-year-old bulls seem to be not as susceptible as older bulls in natural service (Clark et al., 1974).

[1] Council Directive 88/407/EEC of 14 June 1988 laying down the animal health requirements applicable to intra- Community trade in and imports of deep-frozen semen of domestic animals of the bovine species. OJ L 194, 22.7.1988, p. 10–23.

[2] Council Directive 2003/43/EC of 26 May 2003 amending Directive 88/407/EEC laying down the animal health requirements applicable to intra-Community trade in and imports of semen of domestic animals of the bovine species. OJ L 143, 11.6.2003, p. 23–32.

[3] Commission Implementing Decision of 20 September 2011 on imports into the Union of semen of domestic animals of the bovine species (notified under document C(2011) 6426) (Text with EEA relevance) (2011/630/EU).

- Pregnancy examination: All open (i.e. non-pregnant) and aborted cows should be culled or segregated in high-risk sub-herds or groups (Campero and Gottstein, 2007).
- Use of commercial vaccines: Vaccination of all heifers and cows is a good measure to improve reproductive efficiency mainly when risk factors associated to infection cannot be avoided. Inactivated vaccines are available in the Americas, but not in the EU. These vaccines offer an improvement in reproductive efficacy (Kvasnicka et al., 1989, 1992; Edmondson et al., 2017).
- Segregation of the herd in low- and high-risk subherds or groups: In the low-risk subherds, only dams that have recently calved are pregnant for more than 5 months and virgin females must be included. These must be serviced preferably by virgin bulls or by bulls with two negative tests and coming from negative herds. In order to be able to follow the effect of the infection, the same group of females should be serviced with the same male until the disease is controlled (Campero and Gottstein, 2007).
- Limit breeding season: The breeding season should be restricted to less than 4 months to reduce the duration of a possible transmission period as much as possible. In addition, with a shortened breeding season it becomes easier to monitor reproductive performance.

3.1.2. Article 7(b) The impact of diseases

3.1.2.1. Article 7(b)(i) The impact of the disease on agricultural and aquaculture production and other parts of the economy

The level of presence of the disease in the Union

Parameter 1 – Number of MSs where the disease is present

Trichomonosis is limited to beef cattle from some parts of the EU where cattle are breeding by natural service. The presence of the disease has been reported in beef cattle in Spain where the bull prevalence can vary from 4% to 30% (Mendoza-Ibarra et al., 2012, 2013). In addition, the infection has been notified to the OIE in Portugal, France, Hungary and Poland (see Table 3). Currently, there are no monitoring programmes to track the incidence and prevalence of trichomonosis in bovine in Europe. This lack of monitoring and reporting, in combination with inconsistent testing practices, can lead to underestimate the true prevalence and adverse effects of the disease.

The loss of production due to the disease

Parameter 2 – Proportion of production losses (%) by epidemic/endemic situation

Trichomonosis decreases productivity of cattle by inducing reduced conception rates and calf crops, increased days to conception, extended calving seasons, increased costs of replacement bulls, loss of genetic potential due to culling, and lighter weaning weights (Clark et al., 1983b; Rae, 1989; Collantes-Fernández et al., 2014; Michi et al., 2016). It is estimated that beef herds with 20–40% *T. foetus*-positive bulls had a reduction of 14–50% in annual calf crops and an increase of 5–35% in the return to service per cow (Rae, 1989). In a study carried out in a local breed of cattle managed using traditional systems from northern Spain (Collantes-Fernández et al., 2014), *T. foetus* infection increased calving intervals and reduced calf crop. On average, the infected herds required 79 days longer between subsequent calvings and calf production was reduced by 17–18%. The largest losses occur in the first (reduction of 17.6% in calf production) and second (28.6%) years when cows experience primary infection (Clark et al., 1983b). In addition, trichomonosis was reported to cause a 5–12% reduction in weight gain during the sucking/growing period and 4–10% reduction in weaning weights (Michi et al., 2016). Specifically, in a recent study carried out in northern Spain, calves from infected cows were born 35 days later, being nearly 26 kg lighter at weaning (Collantes-Fernández et al., 2014). In previous studies following the resolution of infection in a herd, increases in calving percentages to 90% (Ball et al., 1987) and pregnancy percentages from 74% to 85% (Skirrow et al., 1985) were described.

In the modern dairy industry, it appears that more than 90% of dairy calves are born to AI. For example in Denmark in 2015, around 17% of first parity dairy cows and 7% of older dairy cows were bred using natural service, with the balance using AI and overall for dairy cattle, 90% are inseminated using AI (SEGES, 2016). Similarly in France, 79% of births in dairy herds are by AI (IDELE, 2017). In Ireland, extrapolated data from the Irish Cattle Breeding Federation indicate approximately 45-60% of calves from the dairy herds are sired by AI (ICBF, 2017). According to the German Cattle Breeders' Federation, in Germany around 25% of cattle farms use AI (ADR, 2016), most probably those are

almost all farms keeping dairy cows. In the Netherlands, in 2016 approximately 225 000 natural matings were registered as compared to 1.6 million first AI. In Finland, dairy herds are bred using AI even if in some larger herds also natural mating is used.

Therefore, the economic losses due to trichomonosis in the EU are mostly linked to the beef cattle sector where natural mating is used. Most of the meat bovine herd in Europe is located in four EU Member States: France (34.4%), Spain (15.2%), the UK (12.8%) and Ireland (8.7%). Together, they host more than 70% of the European meat herd (EUROSTAT, online). In France, only 13% of beef cattle are bred from AI (IDELE, 2017). In UK, the majority of beef herds use natural service. AI in beef cows is still uncommon in the UK due to the problems of heat detection and handling for AI (Penny, 2017). In Ireland, only about 23% of calves in beef herds are bred by AI (Agriland, online) and around 80 000 beef stock bulls were present during 2016 (ICBF, 2017). In Italy, data from the National Data Bank on farm animals indicate 17% 'linea vacca-vitello' beef cows representing 900 000 animals are bred by natural service (BDN, online). In Spain, around 95% of beef cattle use natural services (UGAVAN, 2017).

Thus, natural services account for a very considerable proportion of the beef cows born annually in the EU.

3.1.2.2. Article 7(b)(ii) The impact of the disease on human health

Transmissibility between animals and humans

Parameter 1 – Types of routes of transmission between animals and humans

A few cases of *T. foetus* infections have been reported in humans. Routes of infections are unknown. In the most recent case description, the oral route of infection was hypothesised (Suzuki et al., 2016).

Parameter 2 – Incidence of zoonotic cases

No data are available. Only four case reports are available in literature.

Transmissibility between humans

Parameter 3 – Human to human transmission is sufficient to sustain sporadic cases or community-level outbreak

Not applicable – there is no evidence for a transmission of *T. foetus* between humans.

Parameter 4 – Sporadic, endemic, or pandemic potential

A sporadic pattern for *T. foetus* infections in humans is the most likely scenario.

The severity of human forms of the disease

Parameter 5 – Disability-adjusted life year (DALY)

No data available.

The availability of effective prevention or medical treatment in humans

Parameter 6 – Availability of medical treatment and their effectiveness (therapeutic effect and any resistance)

No data available.

Parameter 7 – Availability of vaccines and their effectiveness (reduced morbidity)

No data available. No vaccinations are currently available.

3.1.2.3. Article 7(b)(iii) The impact of the disease on animal welfare

Parameter 1 – Severity of clinical signs at case level and related level and duration of impairment

In cows or heifers following infection at breeding, the parasite multiplication in the reproductive tract causes inflammation of the endometrium, cervical and vaginal mucous membranes (Parsonson et al., 1976; Rhyan et al., 1988; Anderson et al., 1996). Consequently, infection of pregnant cattle can result in early embryonic death, resorption, abortion, pyometra, fetal maceration or infertility (Anderson et al., 1996). Abortions of fetuses typically occur about 2 months gestational age (at > 42 days of gestation with a peak loss at 70–90 days) (Parsonson et al., 1976; BonDurant, 2005).

3.1.2.4. Article 7(b)(iv) The impact of the disease on biodiversity and the environment

Biodiversity

Not applicable – *T. foetus* has not been reported in wildlife species so far.

Environment

Parameter 3 – Capacity of the pathogen to persist in the environment and cause mortality in wildlife

T. foetus is considered incapable of forming cysts and has limited survival capacity outside the host (BonDurant and Honigberg, 1994). However, the formation of pseudocysts has been reported (Pereira-Neves and Benchimol, 2009).

T. foetus was shown to be able to survive in cryopreserved semen (Blackshaw and Beattie, 1955; Clark et al., 1971) and may be present in semen if it is contaminated with preputial fluid during manual collection. Given the resistance of this parasite in fresh, pure or diluted semen, refrigerated and even cryopreserved, there is the possibility of transmission through AI with contaminated semen (BonDurant, 2005). *T. foetus* has not been reported so far in wildlife species, hence no mortality is described.

3.1.3. Article 7(c) Its potential to generate a crisis situation and its potential use in bioterrorism

Parameter 1 – Listed in OIE/CFSPH classification of pathogens

Trichomonosis belongs to the OIE-listed Diseases.
Trichomonosis is not included in the CFSPH classification of pathogens.

Parameter 2 – Listed in the Encyclopaedia of Bioterrorism Defence of Australia Group

T. foetus is not included in the Encyclopaedia of Bioterrorism Defence of Australia Group.

Parameter 3 – Included in any other list of potential bio- agro-terrorism agents

No – no specific list identified.

3.1.4. Article 7(d) The feasibility, availability and effectiveness of the following disease prevention and control measures

3.1.4.1. Article 7(d)(i) Diagnostic tools and capacities

Availability

Parameter 1 – Officially/internationally recognised diagnostic tool, OIE certified

The prescribed test for international trade is the identification of *T. foetus* in bovine samples by cultivation in combination with a microscopic examination and identification of cultured trichomonads. The examination of samples by PCR methods are mentioned as alternatives for diagnosis (OIE, 2017a).

The OIE Terrestrial Manual provides protocols for sampling, sample transportation, transport medium, culture media or conditions, how to read out the culture test and recommendations for PCR analyses.

No diagnostic kits for trichomonosis are listed on the register of diagnostic kits certified by the OIE as validated as fit for purpose. To the best of our knowledge, one *T. foetus* real-time PCR is commercially available (VetMAX™-Gold Trich Detection Kit, Life Technologies).

Other diagnostic procedures, like direct microscopic examination or antibody testing, are not recommended as diagnostic tools due to low diagnostic sensitivity and low specificity.

Effectiveness

Parameter 2 – Se and Sp of diagnostic test

Diagnostic sensitivity (Se) of culture: The diagnostic sensitivity of a single culture test on infected bulls has been estimated at 70–100% (Skirrow et al., 1985; Schönmann et al., 1994; Parker et al., 1999, 2003a,c). A repeated testing of bulls (e.g. three times with intervals of several days) was shown to increase the sensitivity of the cell culture test up to close to 100% (Mukhufhi et al., 2003). Furthermore, consecutive testing over a period of more than 7 months demonstrated infection in

100% of 15 infected bulls in another study (Clark et al., 1971). A sexual rest of bulls for a minimum of about one to two weeks prior to sampling increases sensitivity (Yule et al., 1989).

Also for female cattle (i.e. the examination of cervico-vaginal mucus), the diagnostic sensitivity of a single culture test has been reported in the range of 56–95% (Parsonson et al., 1976; Goodger and Skirrow, 1986; Skirrow and BonDurant, 1988; Kittel et al., 1998). The infection in females is usually cleared within 3 months and it may be difficult to isolate organisms from female cattle in the late stage of their infection, also because of fluctuating concentrations of parasites during this period.

Diagnostic specificity (Sp) of culture: The diagnostic specificity of the culture method is lower than 100% (OIE, 2017a). It is difficult and sometimes not possible to distinguish trichomonad species by morphological criteria. Intestinal trichomonads were observed in virgin bull samples submitted for confirmation of culture diagnosis (BonDurant et al., 1999; Campero et al., 2003; Michi et al., 2016). A subsequent PCR analysis of culture positive samples has been recommended to avoid false positive findings (Parker et al., 2001; Campero et al., 2003).

Diagnostic sensitivity (Se) of PCR: The diagnostic sensitivity of PCR seems to be at least similar to that of culture (Campero et al., 2003; Yao, 2013; OIE, 2017a). PCR can be used to rule out false-positive cultures in bulls, especially in virgin bulls because viability of parasites is not a necessary prerequisite of this technique (Campero et al., 2003; Parker et al., 2003b; Corbeil et al., 2008). Due to PCR inhibiting contaminations or inefficient DNA extraction, analytical sensitivity of PCR might be much lower and is reported to be around ≤ 100 organisms per sample (Mukhufhi et al., 2003; Yao, 2013). Control systems to monitor PCR inhibition are necessary; one commercially available *T. foetus* real-time PCR kit includes an inhibition control (VetMAX™-Gold Trich Detection Kit, Life Technologies).

Diagnostic specificity (Sp) of PCR: Diagnostic PCRs published are targeting the DNA coding for rRNA and flanking regions (rDNA). Because rDNA shows only limited or no differences between trichomonads (Felleisen, 1997), cross-reactivity with DNA of related species are possible. Because *T. foetus* is regarded as host species specific, cross-reaction with trichomonads responsible for feline trichomonosis and *T. suis* infections are of minor importance.

Positive predicive value (PPV) and negative predictive value (NPV) for cell culture and molecular tests have not been established.

Feasibility

Parameter 3 – Type of sample matrix to be tested (blood, tissue, etc.)

For herd diagnosis, it is essential to sample bulls due to the fact that bulls remain persistently infected (Yao, 2013). Preputial material is collected by sheath scraping combined with aspiration (using brushes or insemination pipettes) or by sheath washing. Material is either inoculated into a transport medium, in a transport or cell culture kit or submitted for DNA extraction and diagnostic PCR (OIE, 2017a). Detailed diagnostic procedures have also been described elsewhere (Sager et al., 2007).

Trichomonosis can also be diagnosed in dams although dams are normally only transiently infected; parasites or parasite DNA can be detected in cervico-vaginal mucus collected by aspiration (Sager et al., 2007).

Aborted material may contain *T. foetus* and the analysis of abomasal content is regarded as optimal (BonDurant, 1985; Sager et al., 2007). The sample can be taken with sterile syringe and needle and sent to the laboratory in the same syringe or can be inoculated in transport or cultured medium. *T. foetus* can also be isolated from cotyledons. However, the high degree of contamination of this material limits its use. Isolations can also be made with samples taken from fetal mouth. Swabbing the mucosa of the tongue and roof of the mouth has been recommended (Case and Keefer, 1938).

Optimally, all types of samples should be added to transport media. They should be protected from exposure to daylight and extremes of temperature, which should remain above 5°C and below 38°C (Bryan et al., 1999; OIE, 2017a).

3.1.4.2. Article 7(d)(ii) Vaccination

Availability

Parameter 1 – Types of vaccines available on the market (live, inactivated, DIVA, etc.)

Commercial inactivated vaccines against trichomonosis in bovine prepared from whole organisms are only available in the Americas but not in Europe.

Parameter 2 – Availability/production capacity (per year)

In the US, an inactivated vaccine can be acquired in a monovalent formulation (Trich Guard®, Boehringer Ingelheim, Germany) but also in a polyvalent formulation combined with *Campylobacter foetus* subsp. *venerealis* and *Leptospira* (Trich Guard V5-L®, Boehringer Ingelheim, Germany). In Argentina, an inactivated oily vaccine (Tricovac, Tandil Biological Laboratory, Argentina) containing 5 × 107 trophozoites of *T. foetus* is available.

Effectiveness

Parameter 3 – Field protection as reduced morbidity (as reduced susceptibility to infection and/or to disease)

The main objective of vaccines against trichomonosis in bovine is to eliminate *T. foetus* infection from the reproductive tract before fetal loss occurs (approximately between 50 and 70 days post-infection), without necessarily avoiding parasite colonisation of the epithelium during the first 40 days post-infection (BonDurant et al., 1993; BonDurant, 2005).

In heifers using whole-parasites-based vaccines (Kvasnicka et al., 1989, 1992; Herr et al., 1991; Gault et al., 1995; Anderson et al., 1996; Cobo et al., 2002, 2004), a reduction in the number of infected females, a shorter time of genital infection, and a higher percentage of pregnant females in comparison with the control animals have been reported. In addition, a lower number of services (1.44 vs 1.73 in non-vaccinated, $p < 0.16$), higher percentage of pregnant animals at the first service (66.7% vs 33.3%, $p < 0.05$) (Hudson et al., 1993), with a reduction of embryo/fetal losses of 56.4% (Kvasnicka et al., 1992) was observed. A recent study using TrichGuard® found that only 20% (4/20) of the heifers in the placebo group gave birth to a live calf compared with 50% (10/20) of the vaccinated heifers. Thus, embryonic or fetal loss was detected in 47% (9/19) vaccinated heifers and 71% (10/14) in the placebo group ($p = 0.153$) (Edmondson et al., 2017).

In bulls older than 5 years, the whole cell vaccine lacked a preventive or curative effect (Clark et al., 1983a). Therefore, commercial TrichGuard® vaccine is not indicated for bulls (Herr et al., 1991; BonDurant, 1997).

Parameter 4 – Duration of protection

In heifers, primovaccination and revaccination 2–4 weeks later should precede the breeding season by 4 weeks. Annual revaccination before the breeding season is needed.

Feasibility

Parameter 5 – Way of administration

Inactivated vaccines are administered by the subcutaneous route.

3.1.4.3. Article 7(d)(iii) Medical treatments

Availability

Parameter 1 – Types of drugs available on the market

Various imidazoles were used to treat bulls, but none was both safe and effective. In addition, drug resistant strains have been found (BonDurant, 2005; Rae and Crews, 2006). Currently, there is no approved treatment for cattle infected with *T. foetus* because of concerns regarding toxic residues in meat (BonDurant, 1997).

Parameter 2 – Availability/production capacity (per year)

There is no approved treatment for cattle infected with *T. foetus*.

Effectiveness

Parameter 3 – Therapeutic effects on the field (effectiveness)

Currently, there is no approved treatment for cattle infected with *T. foetus* because of concerns regarding toxic residues in meat (BonDurant, 1997). In addition, drug-resistant *T. foetus* strains have been described (BonDurant, 2005; Rae and Crews, 2006). In the past, ipronidazole was probably the most effective (Skirrow et al., 1985) but, due to its low pH, this drug frequently causes sterile abscesses at injection sites. Moreover, systemic treatment with drugs like metronidazole or

dimetridazole produces adverse side effects and development of resistant populations of trichomonads (Campero et al., 1987).

Feasibility

Parameter 4 – Way of administration

There is no approved treatment for cattle infected with *T. foetus*.

3.1.4.4. Article 7(d)(iv) Biosecurity measures

Availability

Parameter 1 – Available biosecurity measures

For bulls destined for artificial insemination, quarantine and *T. foetus* periodic testing are routinely used. In some areas of the world, as in the EU, specific regulations are applied to bovine semen trade and to regulate the sanitary conditions of the collection centre and the animals (see Section 3.1.1.8).

In herds and areas where it is not possible to introduce AI and natural mating is the normal practice, as in many areas where beef cattle are raised extensively, different policies have been established to control the infection. There is no published information about the existence and results of EU programmes for control of trichomonosis in bovine. On the contrary, in the US, state regulations have endeavoured to control the endemic disease and to minimise economic losses by testing bulls and allowing the importation only of *T. foetus*-free bulls for reproductive purposes while culling of positive bulls (Yao, 2013). In the Argentinian region of La Pampa, with a bovine census close to 4 million head of cattle, more than 80% of the bulls have been tested twice each year. Positive bulls could not be sold to other herds and movement was only allowed to the slaughter house. Under these conditions, a significant decrease in the number of infected herds and animals has been achieved in this region in the last 10 years (Fort et al., 2016). Biosafety recommendations include quarantine and testing replacement bulls and all the measures that help segregating infected from susceptible animals (e.g. avoid communal grazing) (Campero and Gottstein, 2007).

Effectiveness

Parameter 2 – Effectiveness of biosecurity measures in preventing the pathogen introduction

Artificial insemination using semen from *T. foetus* non infected bulls is fully effective in preventing trichomonosis. In fact, trichomonosis in bovine has almost disappeared or is anecdotic reported in dairy cattle industries from western European countries, USA or Canada. However, the disease is still present in many areas of the world, including those reported above, were cattle are raised on communal grazing and natural mating is used.

Quarantine and testing of bulls, including culling of positive animals, and keep fences in a good state is considered very effective in controlling disease in areas where natural mating is practised (Collantes-Fernández et al., 2014).

Feasibility

Parameter 3 – Feasibility of biosecurity measures

Biosecurity measures are feasible both at the international (EU countries) and farm levels.

3.1.4.5. Article 7(d)(v) Restrictions on the movement of animals and products

Availability

Parameter 1 – Available movement restriction measures

In the EU, there are no mandatory regulations for cattle movement regarding trichomonosis. Specific regulations are applied to bovine semen trade and to regulate the sanitary conditions of the collection centre and the animals. Specifically, bulls selected for entry into artificial insemination should be tested in quarantine before admission to the centre and regular testing of animals in service are included as basic measures to avoid the presence and dissemination of trichomonosis (Council Directive 2003/43/EC).

In addition and due to the fact that *T. foetus* may be present in semen, recommendations for the importation of cattle and bulls for breeding can be found in the Terrestrial Animal Health Code. In

particular, emphasis has been placed on the measures applied to bull semen donor health status to avoid dissemination and transmission of the disease (OIE, 2017b).

In the US, there are rules/regulations to reduce spread of the disease in 26 States (Szonyi et al., 2012; Yao, 2013). The core of these regulations is testing bulls. It is also recommended not to introduce cows or heifers of unknown health status during the breeding season. Similar to bulls, heifers and cows must be acquired only from disease-free herds with records of excellence in reproductive performance.

Effectiveness

Parameter 2 – Effectiveness of restriction of animal movement in preventing the between farm spread

Quarantine and testing of bulls, including culling those infected when they are introduced into the herds, is effective in preventing spread of trichomonosis between farms. However, this measure will not be effective enough to control the spread of the disease in endemic areas when other risk factors are present such as cograzing, the use of communal bulls and a high proportion of old bulls (Yao, 2013; Collantes-Fernández et al., 2014).

Regulations applied to bovine semen trade are fully effective in preventing spread of trichomonosis by the use of artificial insemination.

Feasibility

Parameter 3 – Feasibility of restriction of animal movement

Restrictions of animal movement and products are feasible both at the international (EU countries) and farm levels.

3.1.4.6. Article 7(d)(vi) Killing of animals

Availability

Parameter 1 – Available methods for killing animals

One of the most effective measures for controlling trichomonosis in beef herds practising natural mating is the identification and culling of infected bulls (Rae and Crews, 2006).

Effectiveness

Parameter 2 – Effectiveness of killing animals (at farm level or within the farm) for reducing/stopping spread of the disease

Culling of infected bulls is effective in reducing/interrupting spread of the disease. However, as it has been mentioned earlier, this measure will not be effective enough in endemic areas when other risk factors are present such as cograzing, the use of communal bulls and a high proportion of old bulls. A comparison of the prevalence of *T. foetus* infection after testing and culling of infected bulls showed a sustainable reduction of *T. foetus* herd prevalence in an endemic area. Nevertheless, in the second year after starting the study, the herd prevalence did not decrease, and new cases of infected herds were observed (Collantes-Fernández et al., 2014).

Feasibility

Parameter 3 – Feasibility of killing animals

Culling of infected bulls is feasible both at a regional and at farm level.

3.1.4.7. Article 7(d)(vii) Disposal of carcasses and other relevant animal by-products

Availability

Parameter 1 – Available disposal option

Not applicable – *T. foetus* is only transmitted by venereal route and the parasite is considered incapable of forming cysts; its tenacity is extremely limited under environmental conditions and carcasses or by-products represent no source of infection (BonDurant and Honigberg, 1994).

Effectiveness

Parameter 2 – Effectiveness of disposal option

Not applicable – carcasses or by-products represent no source of infection.

Feasibility

Parameter 3 – Feasibility of disposal option

Not applicable – carcasses or by-products represent no source of infection.

3.1.5. Article 7(e) The impact of disease prevention and control measures

3.1.5.1. Article 7(e)(i) The direct and indirect costs for the affected sectors and the economy as a whole

Parameter 1 – Cost of control (e.g. treatment/vaccine, biosecurity)

No data are available concerning costs of control, since it is difficult to quantify and will depend greatly on the size of the premise and the control method selected.

Parameter 2 – Cost of eradication (culling, compensation)

There is no compensation for culling of infected bulls. The cost of culling of infected bulls will depend on the bull carcass weight, cull price data from the local sales yard, transport cost to slaughterhouse and bull replacement costs. Culling/replacement bull cost was estimated about EUR 735 in a Spanish local breed (Collantes-Fernández et al., 2014).

Parameter 3 – Cost of surveillance and monitoring

Cost of bull testing will vary depending on the laboratory and veterinary service fees. In a recent study, trichomonad testing was estimated to cost about EUR 100/bull, including personnel costs (Collantes-Fernández et al., 2014). In Colorado cattle herds, a producer who has a 100 cow herd uses one bull per 20 cows and lives 120 km from a veterinary clinic will incur about EUR 274 to test all of the herd bulls for trichomonosis (Striegel et al., 2009).

Parameter 6 – Trade loss (bans, embargos, sanctions) by animal product

Not applicable.

Parameter 7 – Importance of the disease for the affected sector (% loss or € lost compared to business amount of the sector)

In a study carried out in a local breed of cattle managed using traditional systems from northern Spain (Collantes-Fernández et al., 2014), *T. foetus* infection reduced income by 70% in herds primarily as a result of prolonged calving intervals and calf crop reduction. Elongation of the calving interval translates into an excess of days open, which causes losses about of 33% due to the cost of maintaining open cows. The cost of a delay of 79 days could result in losses of approximately EUR 126 per cow. Calves from infected cows would be born 35 days later, being nearly 26 kg lighter at weaning. Consequently, a producer could be losing nearly EUR 53 (~ 16.3%) for every late calf born in a single season (Collantes-Fernández et al., 2014). In an American study, trichomonosis was reported to cause a 4–10% reduction in monetary returns per calf born and 5–35% reduction in financial returns per cow, compared to those exposed to a non-infected bull (Michi et al., 2016).

3.1.5.2. Article 7(e)(ii) The societal acceptance of disease prevention and control measures

Considering the consequences of infection, following elimination of the infected bulls, an improvement in reproductive performance was observed, and the reproductive data were equivalent to those recorded in the non-infected herds, so this result would be anticipated to be socially very acceptable (Collantes-Fernández et al., 2014). In previous studies following the resolution of infection in a herd, increases in calving percentages to 90% (Ball et al., 1987) and pregnancy percentages from 74% to 85% (Skirrow et al., 1985) were described.

3.1.5.3. Article 7(e)(iii) The welfare of affected subpopulations of kept and wild animals

Parameter 1 – Welfare impact of control measures on domestic animals

The lack of effective vaccines and treatments in bulls may be a significant constraint.

Parameter 2 – Wildlife depopulation as control measure

Not applicable – *T. foetus* has not been reported so far in wildlife species.

3.1.5.4. Article 7(e)(iv) The environment and biodiversity

Environment

Parameter 1 – Use and potential residuals of biocides or medical drugs in environmental compartments (soil, water, feed, manure)

Not applicable.

Biodiversity

Parameter 2 – Mortality in wild species

Not applicable – *T. foetus* has not been reported so far in wildlife species.

3.2. Assessment according to Article 5 criteria

This section presents the results of the expert judgement on the criteria of Article 5 of the AHL about trichomonosis (Table 4). The expert judgement was based on Individual and Collective Behavioural Aggregation (ICBA) approach described in detail in the opinion on the methodology (EFSA AHAW Panel, 2017). Experts have been provided with information of the disease fact-sheet mapped into Article 5 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 5, and the reasoning supporting their judgement.

The minimum number of judges in the judgement was 12. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions, see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

Table 4: Outcome of the expert judgement on the Article 5 criteria for trichomonosis

Criteria to be met by the disease: According to AHL, a disease shall be included in the list referred to in point (b) of paragraph 1 of Article 5 if it has been assessed in accordance with Article 7 and meets all of the following criteria		**Final outcome**
A(i)	The disease is transmissible	Y
A(ii)	Animal species are either susceptible to the disease or vectors and reservoirs thereof exist in the Union	Y
A(iii)	The disease causes negative effects on animal health or poses a risk to public health due to its zoonotic character	Y
A(iv)	Diagnostic tools are available for the disease	Y
A(v)	Risk-mitigating measures and, where relevant, surveillance of the disease are effective and proportionate to the risks posed by the disease in the Union	Y
At least one criterion to be met by the disease: In addition to the criteria set out above at points A(i)–A(v), the disease needs to fulfil at least one of the following criteria		
B(i)	The disease causes or could cause significant negative effects in the Union on animal health, or poses or could pose a significant risk to public health due to its zoonotic character	Y
B(ii)	The disease agent has developed resistance to treatments and poses a significant danger to public and/or animal health in the Union	N
B(iii)	The disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union	Y
B(iv)	The disease has the potential to generate a crisis or the disease agent could be used for the purpose of bioterrorism	N
B(v)	The disease has or could have a significant negative impact on the environment, including biodiversity, of the Union	N

Colour code: green = consensus (Yes/No).

3.2.1. Outcome of the assessment of trichomonosis according to criteria of Article 5(3) of the AHL on its eligibility to be listed

As from the legal text of the AHL, a disease is considered eligible to be listed as laid down in Article 5 if it fulfils all criteria of the first set from A(i) to A(v) and at least one of the second set of criteria from B(i) to B(v). According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'. According to the results shown in Table 4, trichomonosis complies with all criteria of the first set and with two criteria of the second set, therefore it is considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL.

3.3. Assessment according to Article 9 criteria

This section presents the results of the expert judgement on the criteria of Annex IV referring to categories as in Article 9 of the AHL about trichomonosis (Tables 5, 6, 7, 8 and 9). The expert judgement was based on ICBA approach described in detail in the opinion on the methodology. Experts have been provided with information of the disease fact-sheet mapped into Article 9 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 9 and the reasoning supporting their judgement.

The minimum number of judges in the judgement was 12. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions, see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

Table 5: Outcome of the expert judgement related to the criteria of section 1 of Annex IV (category A of Article 9) for trichomonosis (CI: current impact; PI: potential impact)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Final outcome**
1	The disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present only in a very limited part of the territory of the Union	N
2.1	The disease is highly transmissible	N
2.2	There are possibilities of airborne or waterborne or vector-borne spread	N
2.3	The disease affects multiple species of kept and wild animals OR single species of kept animals of economic importance	Y
2.4	The disease may result in high morbidity and significant mortality rates	N
At least one criterion to be met by the disease: In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, including epidemic or pandemic potential OR possible significant threats to food safety	N
4(CI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	NC
4(PI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	Y
5(a)(CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(a)(PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)(CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N
5(b)(PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N
5(c)(CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(c)(PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(d)(CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N
5(d)(PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N

Colour code: green = consensus (Yes/No), yellow = non-consensus (NC).

Table 6: Outcome of the expert judgement related to the criteria of section 2 of Annex IV (category B of Article 9) for trichomonosis (CI: current impact; PI: potential impact)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Final outcome**
1	The disease is present in the whole OR part of the Union territory with an endemic character AND (at the same time) several Member States or zones of the Union are free of the disease	N
2.1	The disease is moderately to highly transmissible	Y
2.2	There are possibilities of airborne or waterborne or vector-borne spread	N
2.3	The disease affects single or multiple species	Y
2.4	The disease may result in high morbidity with in general low mortality	N
At least one criterion to be met by the disease: In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, including epidemic potential OR possible significant threats to food safety	N
4(CI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	NC
4(PI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	Y
5(a)(CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(a)(PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)(CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N
5(b)(PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N
5(c)(CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(c)(PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(d)(CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N
5(d)(PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N

Colour code: green = consensus (Yes/No), yellow = non-consensus (NC).

Table 7: Outcome of the expert judgement related to the criteria of section 3 of Annex IV (category C of Article 9) for trichomonosis (CI: current impact; PI: potential impact)

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Final outcome**
1	The disease is present in the whole OR part of the Union territory with an endemic character	Y
2.1	The disease is moderately to highly transmissible	Y
2.2	The disease is transmitted mainly by direct or indirect transmission	Y
2.3	The disease affects single or multiple species	Y
2.4	The disease usually does not result in high morbidity and has negligible or no mortality AND often the most observed effect of the disease is production loss	Y
At least one criterion to be met by the disease: In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, or possible significant threats to food safety	N

4(CI)	The disease has a significant impact on the economy of parts of the Union, mainly related to its direct impact on certain types of animal production systems	NC
4(PI)	The disease has a significant impact on the economy of parts of the Union, mainly related to its direct impact on certain types of animal production systems	Y
5(a)(CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(a)(PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)(CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N
5(b)(PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	N
5(c)(CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(c)(PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	N
5(d)(CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N
5(d)(PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	N

Colour code: green = consensus (Yes/No), yellow = non-consensus (NC).

Table 8: Outcome of the expert judgement related to the criteria of section 4 of Annex IV (category D of Article 9) for trichomonosis

Criteria to be met by the disease: The disease needs to fulfil all of the following criteria		**Final outcome**
D	The risk posed by the disease in question can be effectively and proportionately mitigated by measures concerning movements of animals and products in order to prevent or limit its occurrence and spread	Y
The disease fulfils criteria of sections 1, 2, 3 or 5 of Annex IV of AHL		Y

Colour code: green = consensus (Yes/No).

Table 9: Outcome of the expert judgement related to the criteria of section 5 of Annex IV (category E of Article 9) for trichomonosis

Diseases in category **E need to fulfil criteria of sections 1, 2 or 3 of Annex IV of AHL** and/or the following:		**Final outcome**
E	Surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment (If a disease fulfils the criteria as in Article 5, thus being eligible to be listed, consequently category E would apply)	Y

Colour code: green = consensus (Yes/No).

3.3.1. Non-consensus-questions

This section displays the assessment related to each criterion of Annex IV referring to the categories of Article 9 of the AHL where no consensus was achieved in form of tables (Table 10). The proportion of Y, N or 'na' answers is reported, followed by the list of different supporting views for each answer.

Table 10: Outcome of the expert judgement related to criterion 4(CI) of Article 9

Question		Final outcome	Response		
			Y (%)	N (%)	Na (%)
4 (cat. A, B)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	NC	25	75	0
4 (cat. C)	The disease has a significant impact on the economy of parts of the Union, mainly related to its direct impact on certain types of animal production systems	NC	58	42	0

NC: non-consensus; number of judges: 12.

Reasoning supporting the judgement

Supporting Yes for 4 (cat. A, B):

- Trichomonosis in bovine decreases productivity of cattle by inducing reduced conception rates and calf crops, increased days to conception, extended calving seasons, increased costs of replacement bulls, loss of genetic potential due to culling and lighter weaning weights. This has an impact on the economy of the Union, especially if the infection is present in countries with extensive beef production.

Supporting No for 4 (cat. A, B):

- In EU countries, the disease presence has dramatically decreased in recent years or even eradicated (in dairy cattle). The impact of the disease is limited to some areas of the EU where beef cattle are breeding by natural service. It is therefore not significant at EU level.

Supporting Yes for 4 (cat. C):

- The impact on the EU economy is significant in some areas of the EU where in extensive beef breeding production systems the use of AI is often impractical and thus natural service is practised.

Supporting No for 4 (cat. C):

- The disease affects a certain type of production system, but the impact is not significant on the economy at EU level.

3.3.2. Outcome of the assessment of criteria in Annex IV for trichomonosis for the purpose of categorisation as in Article 9 of the AHL

As from the legal text of the AHL, a disease is considered fitting in a certain category (A, B, C, D or E corresponding to point (a) to point (e) of Article 9(1) of the AHL) if it is eligible to be listed for Union intervention as laid down in Article 5(3) and fulfils all criteria of the first set from 1 to 2.4 and at least one of the second set of criteria from 3 to 5(d) as shown in Tables 5–9. According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'. With respect to different type of impact where the assessment is divided into current and potential impact, a criterion will be considered fulfilled if at least one of the two outcomes is 'Y' and, in case of no 'Y', the assessment is inconclusive if at least one outcome is 'NC'.

A description of the outcome of the assessment of criteria in Annex IV for trichomonosis for the purpose of categorisation as in Article 9 of the AHL is presented in Table 11.

Table 11: Outcome of the assessment of criteria in Annex IV for trichomonosis for the purpose of categorisation as in Article 9 of the AHL

Category	Article 9 criteria										
	1° set of criteria					2° set of criteria					
	1	2.1	2.2	2.3	2.4	3	4	5a	5b	5c	5d
	Geographical distribution	Transmissibility	Routes of transmission	Multiple species	Morbidity and mortality	Zoonotic potential	Impact on economy	Impact on society	Impact on animal welfare	Impact on environment	Impact on biodiversity
A	N	N	N	Y	N	N	Y	N	N	N	N
B	N	Y	N	Y	N	N	Y	N	N	N	N
C	Y	Y	Y	Y	Y	N	Y	N	N	N	N
D	Y										
E	Y										

According to the assessment here performed, trichomonosis complies with the following criteria of the sections 1–5 of Annex IV of the AHL for the application of the disease prevention and control rules referred to in points (a)–(e) of Article 9(1):

1) To be assigned to category A, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment trichomonosis complies only with criterion 2.3. To be eligible for category A, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and trichomonosis complies with criterion 4, but not with criteria 3, 5a, 5b, 5c and 5d.
2) To be assigned to category B, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment trichomonosis complies with criteria 2.1 and 2.3, but not with criteria 1, 2.2 and 2.4. To be eligible for category B, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and trichomonosis complies with criterion 4 but not with criteria 3, 5a, 5b, 5c and 5d.
3) To be assigned to category C, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment trichomonosis complies with all of them. To be eligible for category C, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and trichomonosis complies with criterion 4 but not with criteria 3, 5a, 5b, 5c and 5d.
4) To be assigned to category D, a disease needs to comply with criteria of sections 1, 2, 3 or 5 of Annex IV of the AHL and with the specific criterion D of section 4, with which trichomonosis complies.
5) To be assigned to category E, a disease needs to comply with criteria of sections 1, 2 or 3 of Annex IV of the AHL and/or the surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment. The latter is applicable if a disease fulfils the criteria as in Article 5, with which trichomonosis complies.

3.4. Assessment of Article 8

This section presents the results of the assessment on the criteria of Article 8(3) of the AHL about trichomonosis. The Article 8(3) criteria are about animal species to be listed, as it reads below:

'3. Animal species or groups of animal species shall be added to this list if they are affected or if they pose a risk for the spread of a specific listed disease because:

a) they are susceptible for a specific listed disease or scientific evidence indicates that such susceptibility is likely; or
b) they are vector species or reservoirs for that disease, or scientific evidence indicates that such role is likely'.

For this reason the assessment on Article 8 criteria is based on the evidence as extrapolated from the relevant criteria of Article 7, i.e. the ones related to susceptible and reservoir species or routes of transmission, which cover also possible role of biological or mechanical vectors.[4] According to the mapping, as presented in Table 5, Section 3.2 of the scientific opinion on the ad hoc methodology (EFSA AHAW Panel, 2017), the main animal species to be listed for trichomonosis according to the criteria of Article 8(3) of the AHL are as displayed in Table 12.

Table 12: Main animal species to be listed for trichomonosis according to criteria of Article 8 (source: data reported in Section 3.1.1.1)

	Class	Order	Family	Genus/species
Susceptible	Mammalia	Artiodactyla	Bovidae	Cattle (*Bos taurus*), zebu (*Bos taurus indicus*)
Reservoir	Mammalia	Artiodactyla	Bovidae	Cattle (*Bos taurus*), zebu (*Bos taurus indicus*)
Vectors	None			

4. Conclusions

TOR 1: *for each of those diseases an assessment, following the criteria laid down in Article 7 of the AHL, on its eligibility of being listed for Union intervention as laid down in Article 5(3) of the AHL;*

- According to the assessment here performed, trichomonosis complies with all criteria of the first set and with two criteria of the second set and therefore can be considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL.

TOR 2a: *for each of the diseases which was found eligible to be listed for Union intervention, an assessment of its compliance with each of the criteria in Annex IV to the AHL for the purpose of categorisation of diseases in accordance with Article 9 of the AHL;*

- According to the assessment here performed, trichomonosis meets the criteria as in sections 3, 4 and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (c), (d) and (e) of Article 9(1) of the AHL.

TOR 2b: *for each of the diseases which was found eligible to be listed for Union intervention, a list of animal species that should be considered candidates for listing in accordance with Article 8 of the AHL.*

- According to the assessment here performed, the animal species that can be considered to be listed for trichomonosis according to Article 8(3) of the AHL is cattle as susceptible and reservoir, as reported in Table 12 in Section 3.4 of the present document.

References

ADR (Arbeitsgemeinschaft Deutscher Rinderzüchter e.V.), 2016. Das Wichtigste in Kürze 2015. Available online: http://www.adr-web.de/services/files/jahresbericht/jahresbericht-2016/Das%20Wichtigste%20in%20K%C3%BCrze%202015.pdf

Agnew DW, Corbeil LB, Munson L, Byrne BA and BonDurant RH, 2008. A pregnant mouse model for bovine Tritrichomonas foetus infection. Veterinary Pathology, 45, 849–864.

Agriland, online. Only 23% of beef calves are born to an AI sire. Available online: http://www.agriland.ie/farming-news/only-23-of-beef-calves-are-born-to-an-ai-sire/ [Accessed: 18 September 2017]

Alexander GI, 1953. An outbreak of bovine trichomoniasis in Queensland and its control. Australian Veterinary Journal, 29, 61–66.

[4] A vector is a living organism that transmits an infectious agent from an infected animal to a human or another animal. Vectors are frequently arthropods. Biological vectors may carry pathogens that can multiply within their bodies and be delivered to new hosts, usually by biting. In mechanical vectors, the pathogens do not multiply within the vector, which usually remains infected for shorter time than in biological vectors.

Anderson ML, BonDurant RH, Corbeil RR and Corbeil LB, 1996. Immune and inflammatory responses to reproductive tract infection with *Tritrichomonas foetus* in immunized and control heifers. The Journal of Parasitology, 82, 594–600.
Ball L, Dargatz DA, Cheney JM and Mortimer RG, 1987. Control of venereal disease in infected herds. The Veterinary Clinics of North America: Food Animal Practice, 3, 561–574.
Barbeito C, Woudwyk M, Cacciato C, Soto P, Portiansky E, Catena M, Echavarria H, Gimeno E and Monteavaro C, 2008. *Tritrichomonas foetus*: experimental infection in pregnant BALB/c mice. Experimental Parasitology, 120, 156–160.
BDN (National Data Bank), online. BDN dell'Anagrafe Zootecnica istituita dal Ministero della Salute presso il CSN dell'Istituto "G. Caporale" di Teramo. Available online: http://statistiche.izs.it/portal/page?_pageid=73,12918&_dad=portal&_schema=PORTAL&op=elenco_rep&p_report=plet_rep_bov&p_titolo=Bovini%20e%20Bufalini [Accessed: 21 September 2017]
Bernasconi C, Bodmer M, Doherr MG, Janett F, Thomann A, Spycher C, Iten C, Hentrich B, Gottstein B, Müller N and Frey CF, 2014. *Tritrichomonas foetus*: prevalence study in naturally mating bulls in Switzerland. Veterinary Parasitology, 200, 289–294.
Bielanski A, Ghazi DF and Phipps-Toodd B, 2004. Observations on the fertilization and development of preimplantation bovine embryos in vitro in the presence of *Tritrichomonas foetus*. Theriogenology, 61, 821–829.
Blackshaw AW and Beattie HER, 1955. The preservation of *Trichomonas foetus* at -79°C. Australian Veterinary Journal, 31, 214–216.
BonDurant RH, 1985. Diagnosis, treatment, and control of bovine trichomoniasis. Compendium: Continuing Education for Veterinarians, 7, 179–188.
BonDurant RH, 1997. Pathogenesis, diagnosis, and management of trichomoniasis in cattle. The Veterinary Clinics of North America: Food Animal Practice, 13, 345–361.
BonDurant RH, 2005. Venereal diseases of cattle: natural history, diagnosis, and the role of vaccines in their control. The Veterinary Clinics of North America: Food Animal Practice, 21, 383–408.
BonDurant RH and Honigberg BM, 1994. Trichomonads of veterinary importance. In: Kreier JP (ed.). *Parasitic Protozoa*, 2nd Edition. Academic Press, New York, New York, USA. pp. 111–188.
BonDurant RH, Anderson ML, Blanchard P, Hird D, Danaye-Elmi C, Palmer C, Sischo WM, Suther D, Utterback W and Weigler BJ, 1990. Prevalence of trichomoniasis among California beef herds. Journal of the American Veterinary Medical Association, 196, 1590–1593.
BonDurant RH, Corbeil RR and Corbeil LB, 1993. Immunization of virgin cows with surface antigen TF1.17 of *Tritrichomonas foetus*. Infection and Immunity, 61, 1385–1394.
BonDurant RH, Gajadhar A and Campero CM, 1999. Preliminary characterization of a *Tritrichomonas foetus*-like protozoan isolated from preputial smegma of virgin bulls. The Bovine Practitioner, 33, 124–127.
Bryan LA, Campbell JR and Gajadhar AA, 1999. Effects of temperature on the survival of *Tritrichomonas foetus* in transport, Diamond's and InPouch TF media. Veterinary Record, 144, 227–232.
Campero C and Gottstein B, 2007. Control measures, tritrichomoniasis. In: Ortega-Mora LM, Gottstein B, Conraths FJ and Buxton D (eds.). *Protozoal Abortion in Farm Ruminants: Guidelines for Diagnosis and Control*. CAB International, Wallingford, Oxfordshire, UK. pp. 290–301.
Campero CM, Ballabene NC, Cipolla AC and Zamora AS, 1987. Dual infection of bulls with campylobacteriosis and trichomoniasis: treatment with dimetridazole chlorhydrate. Australian Veterinary Journal, 64, 320–321.
Campero CM, Hirst RG, Ladds PW, Vaughan JA, Emery DL and Watson DL, 1990. Measurement of antibody in serum and genital fluids of bulls by ELISA after vaccination and challenge with *Tritrichomonas foetus*. Australian Veterinary Journal, 67, 175–178.
Campero CM, Rodriguez Dubra C, Bolondi A, Cacciato C, Cobo E, Perez S, Odeon A, Cipolla A and BonDurant RH, 2003. Two-step (culture and PCR) diagnostic approach for differentiation of non-*T. foetus* trichomonads from genitalia of virgin beef bulls in Argentina. Veterinary Parasitology, 112, 167–175.
Case CH and Keefer WO, 1938. Some ways to detect and prevent the spread of trichomoniasis in cattle. Journal of the American Veterinary Medical Association, 93, 239–240.
Casteriano A, Molini U, Kandjumbwa K, Khaiseb S, Frey CF and Slapeta J, 2016. Novel genotype of *Tritrichomonas foetus* from cattle in Southern Africa. Parasitology, 143, 1954–1959.
Christensen HR, Clark BL and Parsonson IM, 1977. Incidence of *Tritrichomonas foetus* in young replacement bulls following introduction into an infected herd. Australian Veterinary Journal, 53, 132–134.
Clark BL, White MB and Banfield JC, 1971. Diagnosis of *Trichomonas foetus* infection in bulls. Australian Veterinary Journal, 47, 181–183.
Clark BL, Parsonson IM and Dufty JH, 1974. Letter: infection of bulls with *Tritrichomonas foetus* through mating with infected heifers. Australian Veterinary Journal, 50, 180.
Clark BL, Dufty JH and Parsonson IM, 1977. Studies on the transmission of *Tritrichomonas foetus*. Australian Veterinary Journal, 53, 170–172.
Clark BL, Dufty JH and Parsonson IM, 1983a. Immunisation of bulls against trichomoniasis. Australian Veterinary Journal, 60, 178–179.
Clark BL, Dufty JH and Parsonson IM, 1983b. The effect of *Tritrichomonas foetus* infection on calving rates in beef cattle. Australian Veterinary Journal, 60, 71–74.

Cobo ER, Cano D, Rossetti O and Campero CM, 2002. Heifers immunized with whole-cell and membrane vaccines against *Tritrichomonas foetus* and naturally challenged with an infected bull. Veterinary Parasitology, 109, 169–184.

Cobo ER, Morsella C, Cano D, Cipolla A and Campero CM, 2004. Immunization in heifers with dual vaccines containing *Tritrichomonas foetus* and *Campylobacter fetus* antigens using systemic and mucosal routes. Theriogenology, 62, 1367–1382.

Collantes-Fernández E, Mendoza-Ibarra JA, Pedraza-Diaz S, Rojo-Montejo S, Navarro-Lozano V, Sanchez-Sanchez R, Ruíz-Santa-Quiteria JA, Ortega-Mora LM and Osoro K, 2014. Efficacy of a control program for bovine trichomonosis based on testing and culling infected bulls in beef cattle managed under mountain pastoral systems of Northern Spain. Veterinary Journal, 200, 140–145.

Corbeil LB, Campero CM, Van Hoosear K and Bondurant RH, 2008. Detection of trichomonad species in the reproductive tracts of breeding and virgin bulls. Veterinary Parasitology, 154, 226–232.

Duboucher C, Caby S, Dufernez F, Chabe M, Gantois N, Delgado-Viscogliosi P, Billy C, Barre E, Torabi E, Capron M, Pierce RJ, Dei-Cas E and Viscogliosi E, 2006. Molecular identification of *Tritrichomonas foetus*-like organisms as coinfecting agents of human *Pneumocystis pneumonia*. Journal of Clinical Microbiology, 44, 1165–1168.

Eaglesome MD and Garcia MM, 1997. Disease risks to animal health from artificial insemination with bovine semen. Revue scientifique et technique (International Office of Epizootics), 16, 215–225.

Edmondson MA, Joiner KS, Spencer JA, Riddell KP, Rodning SP, Gard JA and Givens MD, 2017. Impact of a killed *Tritrichomonas foetus* vaccine on clearance of the organism and subsequent fertility of heifers following experimental inoculation. Theriogenology, 90, 245–251.

Effinger L, Peddireddi L, Simunich M, Oberst R, O'Connell C and Leyva-Baca I, 2014. Pooling of cultured samples and comparison of multistate laboratory workflows with the MagMAX sample preparation system and VetMAX quantitative polymerase chain reaction reagents for detection of *Tritrichomonas foetus*-colonized bulls. Journal of Veterinary Diagnostic Investigation, 26, 72–87.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More S, Bøtner A, Butterworth A, Calistri P, Depner K, Edwards S, Garin-Bastuji B, Good M, Gortázar Schmidt C, Michel V, Miranda MA, Nielsen SS, Raj M, Sihvonen L, Spoolder H, Stegeman JA, Thulke H-H, Velarde A, Willeberg P, Winckler C, Baldinelli F, Broglia A, Candiani D, Gervelmeyer A, Zancanaro G, Kohnle L, Morgado J and Bicout D, 2017. Scientific opinion on an ad hoc method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law. EFSA Journal 2017;15(5):4783, 42 pp. https://doi.org/10.2903/j.efsa.2017.4783

Erasmus JA, De Wet JA, Van der Merwe HE and Pienaar GC, 1989. Bovine trichomoniasis in the north Western Cape province, western transvaal and the orange free state. Journal of the South African Veterinary Association, 60, 51–52.

EUROSTAT, online. Meat production statistics. Available online: http://ec.europa.eu/eurostat/statistics-explained/index.php/Meat_production_statistics#Further_Eurostat_information [Accessed: 18 September 2017]

Felleisen RS, 1997. Comparative sequence analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. Parasitology, 115, 111–119.

Felleisen RS, Lambelet N, Bachmann P, Nicolet J, Müller N and Gottstein B, 1998. Detection of *Tritrichomonas foetus* by PCR and DNA enzyme immunoassay based on rRNA gene unit sequences. Journal of Clinical Microbiology, 36, 513–519.

Flower PJ, Ladds PW, Thomas AD and Watson DL, 1983. An immunopathologic study of the bovine prepuce. Veterinary Pathology, 20, 189–202.

Fort M, Dubié D, Sago A, Goyeneche P, Benini A, Fernández E, Collantes-Fernández E, Sánchez-Sánchez R, Moreno-Gonzalo J and Ortega-Mora LM, 2016. Evaluation of the performance of bovine trichomonosis control program in La Pampa-Argentina. Proceedings of the 21st International Congress ANEMBE, Santiago de Compostela, Spain.

Frey CF and Müller N, 2012. *Tritrichomonas*–systematics of an enigmatic genus. Molecular and Cellular Probes, 26, 132–136.

Frey CF, Schild M, Hemphill A, Stünzi P, Müller N, Gottstein B and Burgener IA, 2009. Intestinal *Tritrichomonas foetus* infection in cats in Switzerland detected by in vitro cultivation and PCR. Parasitology Research, 104, 783–788.

Gault RA, Kvasnicka WG, Hanks D, Hanks M and Hall MR, 1995. Specific antibodies in serum and vaginal mucus of heifers inoculated with a vaccine containing *Tritrichomonas foetus*. American Journal of Veterinary Research, 56, 454–459.

Gay JM, Ebel ED and Kearley WP, 1996. Commingled grazing as a risk factor for trichomonosis in beef herds. Journal of the American Veterinary Medical Association, 209, 643–646.

Goodger WJ and Skirrow SZ, 1986. Epidemiologic and economic analyses of an unusually long epizootic of trichomoniasis in a large California dairy herd. Journal of the American Veterinary Medical Association, 189, 772–776.

Gookin JL, Breitschwerdt EB, Levy MG, Gager RB and Benrud JG, 1999. Diarrhea associated with trichomonosis in cats. Journal of the American Veterinary Medical Association, 215, 1450–1454.

Gookin JL, Levy MG, Law JM, Papich MG, Poore MF and Breitschwerdt EB, 2001. Experimental infection of cats with *Tritrichomonas foetus*. American Journal of Veterinary Research, 62, 1690–1697.

Gookin JL, Birkenheuer AJ, Breitschwerdt EB and Levy MG, 2002. Single-tube nested PCR for detection of *Tritrichomonas foetus* in feline feces. Journal of Clinical Microbiology, 40, 4126–4130.
Gookin JL, Birkenheuer AJ, St John V, Spector M and Levy MG, 2005. Molecular characterization of trichomonads from feces of dogs with diarrhea. The Journal of Parasitology, 91, 939–943.
Grabensteiner E and Hess M, 2006. PCR for the identification and differentiation of *Histomonas meleagridis*, *Tetratrichomonas gallinarum* and *Blastocystis* spp. Veterinary Parasitology, 142, 223–230.
Grahn RA, BonDurant RH, van Hoosear KA, Walker RL and Lyons LA, 2005. An improved molecular assay for *Tritrichomonas foetus*. Veterinary Parasitology, 127, 33–41.
Grellet A, Bickel T, Polack B, Boogaerts C, Casseleux G, Biourge V and Grandjean D, 2010. Prevalence of *Tritrichomonas foetus* in puppies from French breeding kennels. Journal of Veterinary Internal Medicine, 24, 1572.
Guven E, Bastem Z, Avcioglu H and Erdem H, 2013. Molecular determination of *Tritrichomonas* spp. in aborted bovine foetuses in Eastern Anatolian Region of Turkey. Veterinary Parasitology, 196, 278–282.
Hampl V, Pavlicek A and Flegr J, 2001. Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. International Journal of Systematic and Evolutionary Microbiology, 51, 731–735.
Hayes DC, Anderson RR and Walker RL, 2003. Identification of trichomonadid protozoa from the bovine preputial cavity by polymerase chain reaction and restriction fragment length polymorphism typing. Journal of Veterinary Diagnostic Investigation, 15, 390–394.
Herr S, Ribeiro LM, Claassen E and Myburgh JG, 1991. A reduction in the duration of infection with *Tritrichomonas foetus* following vaccination in heifers and the failure to demonstrate a curative effect in infected bulls. The Onderstepoort Journal of Veterinary Research, 58, 41–45.
Ho MS, Conrad PA, Conrad PJ, LeFebvre RB, Perez E and BonDurant RH, 1994. Detection of bovine trichomoniasis with a specific DNA probe and PCR amplification system. Journal of Clinical Microbiology, 32, 98–104.
Huby-Chilton F, Scandrett BW, Chilton NB and Gajadhar AA, 2009. Detection and identification of *Tetratrichomonas* in a preputial wash from a bull by PCR and SSCP. Veterinary Parasitology, 166, 199–204.
Hudson DB, Ball L, Cheney JM, Mortimer RG, Bowen RA, Marsh DJ and Peetz RH, 1993. Development and testing of a bovine trichomoniasis vaccine. Theriogenology, 39, 929–935.
ICBF (Irish Cattle Breeding Federation), 2017. Beef & Dairy Breed Statistics 2017. ICBF Web Editor, 44 pp. Available online: https://www.icbf.com/wp/?p=9153
IDELE (Insistut de l'Elevage), 2017. Stratégies de conduit de la reproduction des bovins. Vu dans Reproscope, La letter de l'observatoire de la reproduction des bovins en France. IDELE, 2 pp. Available online: http://idele.fr/?eID=cmis_download&oID=workspace://SpacesStore/4f8d06b1-cfbf-43fd-94dc-d824d9a66efc
Jin Y, Schumaker B, Logan J and Yao C, 2014. Risk factors associated with bovine trichomoniasis in beef cattle identified by a questionnaire. Journal of Medical Microbiology, 63, 896–902.
Jongwutiwes S, Silachamroon U and Putaporntip C, 2000. *Pentatrichomonas hominis* in empyema thoracis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 94, 185–186.
Kimsey PB, 1986. Bovine trichomoniasis. In: Morrow DA (ed.). *Current Therapy in Theriogenology*, 2nd Edition. WB Saunders Co, Philadelphia, USA. pp. 275–279.
Kimsey PB, Darien BJ, Kendrick JW and Franti CE, 1980. Bovine trichomoniasis: diagnosis and treatment. Journal of the American Veterinary Medical Association, 177, 616–619.
Kittel DR, Campero C, Van Hoosear KA, Rhyan JC and BonDurant RH, 1998. Comparison of diagnostic methods for detection of active infection with *Tritrichomonas foetus* in beef heifers. Journal of the American Veterinary Medical Association, 213, 519–522.
Kvasnicka WG, Taylor RE, Huang JC, Hanks D, Tronstad RJ, Bosomworth A and Hall MR, 1989. Investigations of the incidence of bovine trichomoniasis in Nevada and of the efficacy of immunizing cattle with vaccines containing *Tritrichomonas foetus*. Theriogenology, 31, 963–971.
Kvasnicka WG, Hanks D, Huang JC, Hall MR, Sandblom D, Chu HJ, Chavez L and Acree WM, 1992. Clinical evaluation of the efficacy of inoculating cattle with a vaccine containing *Tritrichomonas foetus*. American Journal of Veterinary Research, 53, 2023–2027.
Levy MG, Gookin JL, Poore M, Birkenheuer AJ, Dykstra MJ and Litaker RW, 2003. *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the etiologic agent of feline trichomonal diarrhea. The Journal of Parasitology, 89, 99–104.
Lun ZR and Gajadhar AA, 1999. A simple and rapid method for staining *Tritrichomonas foetus* and *Trichomonas vaginalis*. Journal of Veterinary Diagnostic Investigation, 11, 471–474.
Lun ZR, Chen XG, Zhu XQ, Li XR and Xie MQ, 2005. Are *Tritrichomonas foetus* and *Tritrichomonas suis* synonyms? Trends in Parasitology, 21, 122–125.
Mancebo OA, Russo AM, Carabajal LL and Monzon CM, 1995. Persistence of *Tritrichomonas foetus* in naturally infected cows and heifers in Argentina. Veterinary Parasitology, 59, 7–11.
Mardones FO, Perez AM, Martínez A and Carpenter TE, 2008. Risk factors associated with *Tritrichomonas foetus* infection in beef herds in the Province of Buenos Aires, Argentina. Veterinary Parasitology, 153, 231–237.

McCool CJ, Townsend MP, Wolfe SG, Simpson MA, Olm TC, Jayawardhana GA and Carney JV, 1988. Prevalence of bovine venereal disease in the Victoria River District of the Northern Territory: likely economic effects and practicable control measures. Australian Veterinary Journal, 65, 153–156.
McMillen L and Lew AE, 2006. Improved detection of *Tritrichomonas foetus* in bovine diagnostic specimens using a novel probe-based real time PCR assay. Veterinary Parasitology, 141, 204–215.
Mendoza-Ibarra JA, Pedraza-Díaz S, García-Peña FJ, Rojo-Montejo S, Ruíz-Santa-Quiteria JA, San Miguel-Ibáñez E, Navarro-Lozano V, Ortega-Mora LM, Osoro K and Collantes-Fernández E, 2012. High prevalence of *Tritrichomonas foetus* infection in Asturiana de la Montaña beef cattle kept in extensive conditions in Northern Spain. Veterinary Journal, 193, 146–151.
Mendoza-Ibarra JA, Ortega-Mora LM, Pedraza-Díaz S, Rojo-Montejo S, Ruíz-Santa-Quiteria JA, Garcia-Peña FJ, Navarro-Lozano V, Cuevas-Martín Mdel C, Osoro K and Collantes-Fernández E, 2013. Differences in the prevalence of *Tritrichomonas foetus* infection in beef cattle farmed under extensive conditions in northern Spain. Veterinary Journal, 196, 547–549.
Michi AN, Favetto PH, Kastelic J and Cobo ER, 2016. A review of sexually transmitted bovine trichomoniasis and campylobacteriosis affecting cattle reproductive health. Theriogenology, 85, 781–791.
Molina L, Perea J, Meglia G, Angón E and García A, 2013. Spatial and temporal epidemiology of bovine trichomoniasis and bovine genital campylobacteriosis in La Pampa province (Argentina). Preventive Veterinary Medicine, 110, 388–394.
Morin-Adeline V, Fraser ST, Stack C and Slapeta J, 2015. Host origin determines pH tolerance of *Tritrichomonas foetus* isolates from the feline gastrointestinal and bovine urogenital tracts. Experimental Parasitology, 157, 68–77.
Mueller K, Morin-Adeline V, Gilchrist K, Brown G and Slapeta J, 2015. High prevalence of *Tritrichomonas foetus* 'bovine genotype' in faecal samples from domestic pigs at a farm where bovine trichomonosis has not been reported for over 30 years. Veterinary Parasitology, 212, 105–110.
Mukhufhi N, Irons PC, Michel A and Peta F, 2003. Evaluation of a PCR test for the diagnosis of *Tritrichomonas foetus* infection in bulls: effects of sample collection method, storage and transport medium on the test. Theriogenology, 60, 1269–1278.
Murname D, 1959. Studies on the transmission of *Tritrichomonas foetus*. Australian Veterinary Journal, 35, 80–83.
Mutwiri GK and Corbeil LB, 1998. Genital and systemic immune responses in a murine model of *Tritrichomonas foetus* infection. The Journal of Parasitology, 84, 321–327.
NDA (Nebraska Department of Agriculture), online. Trichomoniasis. Available online: http://www.nda.nebraska.gov/animal/diseases/trich/
Nickel DD, Olson ME and Schultz GA, 2002. An improved polymerase chain reaction assay for the detection of *Tritrichomonas foetus* in cattle. The Canadian Veterinary Journal, 43, 213–216.
OIE (World Organization for Animal Health), 2017a. Manual of diagnostic tests and vaccines for terrestrial animals 2017. Available online: http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/
OIE (World Organization for Animal Health), 2017b. Trichomonosis. In: terrestrial animal health code. Available online: http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_trichomonosis.htm
Okamoto S, Wakui M, Kobayashi H, Sato N, Ishida A, Tanabe M, Takeuchi T, Fukushima S, Yamada T and Ikeda Y, 1998. *Trichomonas foetus* meningoencephalitis after allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplantation, 21, 89–91.
Oliveira JM, da Silva GM, Batista Filho AF, de Melo Borges J, de Oliveira PR, Brandespim DF, Mota RA and Pinheiro Jr JW, 2015. Prevalence and risk factors associated with bovine genital campylobacteriosis and bovine trichomonosis in the state of Pernambuco, Brazil. Tropical Animal Health and Production, 47, 549–555.
Ondrak JD, 2016. *Tritrichomonas foetus* prevention and control in cattle. The Veterinary Clinics of North America: Food Animal Practice, 32, 411–423.
Parker S, Campbell J, Ribble C and Gajadhar A, 1999. Comparison of two sampling tools for diagnosis of *Tritrichomonas foetus* in bulls and clinical interpretation of culture results. Journal of the American Veterinary Medical Association, 215, 231–235.
Parker S, Lun ZR and Gajadhar A, 2001. Application of a PCR assay to enhance the detection and identification of *Tritrichomonas foetus* in cultured preputial samples. Journal of Veterinary Diagnostic Investigation, 13, 508–513.
Parker S, Campbell J and Gajadhar A, 2003a. Comparison of the diagnostic sensitivity of a commercially available culture kit and a diagnostic culture test using Diamond's media for diagnosing *Tritrichomonas foetus* in bulls. Journal of Veterinary Diagnostic Investigation, 15, 460–465.
Parker S, Campbell J, McIntosh K and Gajadhar A, 2003b. Diagnosis of trichomoniasis in 'virgin' bulls by culture and polymerase chain reaction. The Canadian Veterinary Journal, 44, 732–734.
Parker S, Campbell J, Ribble C and Gajadhar A, 2003c. Sample collection factors affect the sensitivity of the diagnostic test for *Tritrichomonas foetus* in bulls. Canadian Journal of Veterinary Research, 67, 138–141.
Parsonson IM, Clark BL and Dufty J, 1974. The pathogenesis of *Tritrichomonas foetus* infection in the bull. Australian Veterinary Journal, 50, 421–423.
Parsonson IM, Clark BL and Dufty JH, 1976. Early pathogenesis and pathology of *Tritrichomonas foetus* infection in virgin heifers. Journal of Comparative Pathology, 86, 59–66.

Penny CD, 2017. Synchronisation for Artificial Insemination. Beef Herd Fertility. National Animal Disease Information Service. Available online: http://www.nadis.org.uk/bulletins/beef-herd-fertility/synchronised-breeding-in-beef-herds.aspx

Pereira-Neves A and Benchimol M, 2009. *Tritrichomonas foetus*: budding from multinucleated pseudocysts. Protist, 160, 536–551.

Pérez E, Conrad PA, Hird D, Ortuno A, Chacon J, BonDurant R and Noordhuizen J, 1992. Prevalence and risk-factors for *Trichomonas-fetus* infection in cattle in northeastern Costa-Rica. Preventive Veterinary Medicine, 14, 155–165.

Profizi C, Cian A, Meloni D, Hugonnard M, Lambert V, Groud K, Gagnon AC, Viscogliosi E and Zenner L, 2013. Prevalence of *Tritrichomonas foetus* infections in French catteries. Veterinary Parasitology, 196, 50–55.

Rae DO, 1989. Impact of trichomoniasis on the cow-calf producer's profitability. Journal of the American Veterinary Medical Association, 194, 771–775.

Rae DO and Crews JE, 2006. *Tritrichomonas foetus*. The Veterinary Clinics of North America: Food Animal Practice, 22, 595–611.

Rae DO, Crews JE, Greiner EC and Donovan GA, 2004. Epidemiology of *Tritrichomonas foetus* in beef bull populations in Florida. Theriogenology, 61, 605–618.

Reinmann K, Müller N, Kuhnert P, Campero CM, Leitsch D, Hess M, Henning K, Fort M, Müller J, Gottstein B and Frey CF, 2012. *Tritrichomonas foetus* isolates from cats and cattle show minor genetic differences in unrelated loci ITS-2 and EF-1alpha. Veterinary Parasitology, 185, 138–144.

Rhyan JC, Stackhouse LL and Quinn WJ, 1988. Fetal and placental lesions in bovine abortion due to *Tritrichomonas foetus*. Veterinary Pathology, 25, 350–355.

Rhyan JC, Wilson KL, Wagner B, Anderson ML, BonDurant RH, Burgess DE, Mutwiri GK and Corbeil LB, 1999. Demonstration of *Tritrichomonas foetus* in the external genitalia and of specific antibodies in preputial secretions of naturally infected bulls. Veterinary Pathology, 36, 406–411.

Robinson RA, Lawson B, Toms MP, Peck KM, Kirkwood JK, Chantrey J, Clatworthy IR, Evans AD, Hughes LA, Hutchinson OC, John SK, Pennycott TW, Perkins MW, Rowley PS, Simpson VR, Tyler KM and Cunningham AA, 2010. Emerging infectious disease leads to rapid population declines of common British birds. PLoS ONE, 5, e12215.

Rodning SP, Wolfe DF, Carson RL, Wright JC, Stockdale HD, Pacoli ME, Busby HC and Rowe SE, 2008. Prevalence of *Tritrichomonas foetus* in several subpopulations of Alabama beef bulls. Theriogenology, 69, 212–217.

Ryley JF and Stacey GJ, 1963. Experimental approaches to chemotherapy of trichomoniasis. Parasitology, 53, 303–320.

Sager H, Ferre I, Henning K and Ortega-Mora LM, 2007. Tritrichomoniasis. In: Ortega-Mora LM, Gottstein B, Conraths FJ and Buxton D (eds.). *Protozoal Abortion in Farm Ruminants: Guidelines for Diagnosis and Control*. CAB International, Wallingford, Oxfordshire, UK. pp. 232–262.

Schönmann MJ, BonDurant RH, Gardner IA, Van Hoosear K, Baltzer W and Kachulis C, 1994. Comparison of sampling and culture methods for the diagnosis of *Tritrichomonas foetus* infection in bulls. Veterinary Record, 134, 620–622.

SEGES Avlsværdivurdering Kvæg Team, 2016. Årsstatistik Avl 2015/16. SEGES, Skejby, 158 pp. Available online: https://www.landbrugsinfo.dk/Kvaeg/Avl/Avlsstatistik/Sider/aarsstat_2016.pdf?download=true

Skirrow S, 1987. Identification of trichomonad-carrier cows. Journal of the American Veterinary Medical Association, 191, 553–554.

Skirrow SZ and BonDurant RH, 1988. Bovine trichomoniasis. Veterinary. Bulletin, 58, 594–603.

Skirrow SZ and BonDurant RH, 1990. Induced *Tritrichomonas foetus* infection in beef heifers. Journal of the American Veterinary Medical Association, 196, 885–889.

Skirrow S, BonDurant R, Farley J and Correa J, 1985. Efficacy of ipronidazole against trichomoniasis in beef bulls. Journal of the American Veterinary Medical Association, 187, 405–407.

Slapeta J, Craig S, McDonell D and Emery D, 2010. *Tritrichomonas foetus* from domestic cats and cattle are genetically distinct. Experimental Parasitology, 126, 209–213.

Slapeta J, Müller N, Stack CM, Walker G, Lew-Tabor A, Tachezy J and Frey CF, 2012. Comparative analysis of *Tritrichomonas foetus* (Riedmuller, 1928) cat genotype, *T. foetus* (Riedmuller, 1928) cattle genotype and *Tritrichomonas suis* (Davaine, 1875) at 10 DNA loci. International Journal for Parasitology, 42, 1143–1149.

St Claire MC, Riley LK, Franklin CL, Besch-Williford CL and Hook Jr RR, 1994. Experimentally induced intravaginal *Tritrichomonas foetus* infection in the estrogenized mouse. Laboratory Animal Science, 44, 430–435.

Stockdale H, Rodning S, Givens M, Carpenter D, Lenz S, Spencer J, Dykstra C, Lindsay D and Blagburn B, 2007. Experimental infection of cattle with a feline isolate of *Tritrichomonas foetus*. Journal of Parasitology, 93, 1429–1434.

Striegel N, Ellis R and Deering J, 2009. Trichomoniasis Prevention: The Cost per Cow to Prevent. Fact Sheet No. 1.628. Colorado State University Extension, 3, 4 pp. Available online: http://extension.colostate.edu/docs/pubs/livestk/01628.pdf

Sun Z, Stack C and Slapeta J, 2012. Sequence differences in the diagnostic region of the cysteine protease 8 gene of *Tritrichomonas foetus* parasites of cats and cattle. Veterinary Parasitology, 186, 445–449.

Suzuki J, Kobayashi S, Osuka H, Kawahata D, Oishi T, Sekiguchi K, Hamada A and Iwata S, 2016. Characterization of a human isolate of *Tritrichomonas foetus* (cattle/swine genotype) infected by a zoonotic opportunistic infection. The Journal of Veterinary Medical Science, 78, 633–640.

Szonyi B, Srinath I, Schwartz A, Clavijo A and Ivanek R, 2012. Spatio-temporal epidemiology of *Tritrichomonas foetus* infection in Texas bulls based on state-wide diagnostic laboratory data. Veterinary Parasitology, 186, 450–455.

Tachezy J, Tachezy R, Hampl V, Sedinova M, Vanacova S, Vrlik M, Van Ranst M, Flegr J and Kuldaa J, 2002. Cattle pathogen *Tritrichomonas foetus* (Riedmuller, 1928) and pig commensal *Tritrichomonas suis* (Gruby & Delafond, 1843) belong to the same species. The Journal of Eukaryotic Microbiology, 49, 154–163.

TRACES (TRAde Control and Expert System), online. Available online: https://webgate.ec.europa.eu/sanco/traces/

UGAVAN (Unión de Ganaderos de Vacas Nodrizas), 2017. Personal communication to EFSA, September 2017.

Van der Saag M, McDonell D and Slapeta J, 2011. Cat genotype *Tritrichomonas foetus* survives passage through the alimentary tract of two common slug species. Veterinary Parasitology, 177, 262–266.

Villarroel A, Carpenter TE and BonDurant RH, 2004. Development of a simulation model to evaluate the effect of vaccination against *Tritrichomonas foetus* on reproductive efficiency in beef herds. American Journal of Veterinary Research, 65, 770–775.

Walden HS, Dykstra C, Dillon A, Rodning S, Givens D, Bird R, Newton J and Lindsay D, 2013. A new species of *Tritrichomonas* (Sarcomastigophora: Trichomonida) from the domestic cat (*Felis catus*). Parasitology Research, 112, 2227–2235.

Yang N, Cui X, Qian W, Yu S and Liu Q, 2012. Survey of nine abortifacient infectious agents in aborted bovine fetuses from dairy farms in Beijing, China, by PCR. Acta Veterinaria Hungarica, 60, 83–92.

Yao C, 2013. Diagnosis of *Tritrichomonas foetus*-infected bulls, an ultimate approach to eradicate bovine trichomoniasis in US cattle? Journal of Medical Microbiology, 62, 1–9.

Yao C, 2015. *Tritrichomonas foetus* infections in female beef cattle with abortion in Wyoming, USA. JMM Case Reports, 2, 1–5.

Yao C and Köster LS, 2015. *Tritrichomonas foetus* infection, a cause of chronic diarrhea in the domestic cat. Veterinary Research, 46, 35.

Yao C, Bardsley KD, Litzman EA, Hall ML and Davidson MR, 2011. *Tritrichomonas foetus* infection in beef bull populations in Wyoming. Journal of Bacteriology and Parasitology, 2, 117.

Yule A, Skirrow SZ and Bonduran RH, 1989. Bovine trichomoniasis. Parasitology Today, 5, 373–377.

Zalonis CA, Pillay A, Secor W, Humburg B and Aber R, 2011. Rare case of trichomonal peritonitis. Emerging Infectious Diseases, 17, 1312–1313.

Abbreviations

AHAW	EFSA Panel on Animal Health and Welfare
AHL	Animal Health Law
AI	Artificial insemination
ICBA	Individual and Collective Behavioural Aggregation
ITS	internal transcribed spacer
LSU	large subunit
NPV	negative predictive value
OIE	World Organization for Animal Health
PCR	polymerase chain reaction
PPV	positive predictive value
rDNA	recombinant DNA
RFLP	restriction fragment length polymorphism
rRNA	ribosomal ribonucleic acid
Se	diagnostic sensitivity
Sp	diagnostic specificity
SSCP	single-strand conformation polymorphism
SSU	small subunit
ToR	Terms of Reference

Appendix A – Diagnosis of trichomonosis

Table A.1: PCRs assays for *Trichomonas* detection

Target	Type of PCR	Name of primer	Name of probe (type of probe)	Reported specificity	Reported sensitivity	Remarks	Reference
ITS1/5.8S rRNA/ ITS2	End-point	TFR1, TFR2	NA	Amplifies *T. foetus*, *T. suis*, *T. mobilensis*, *T. vaginalis*, *T. gallinae*, *T. tenax*, *P. hominis* (Felleisen et al., 1998). No amplification of bacterial DNA, or purified bovine genomic DNA	1 or a few protozoa	Also referred to as pan-trichomonad PCR	Felleisen (1997)
ITS1/5.8S rRNA/ ITS2	End-point	TFR3, TFR4	NA	Amplifies *T. foetus*, *T. suis*, *T. mobilensis*	1 or a few protozoa	Often referred to as *T. foetus* specific PCR	Felleisen et al. (1998)
18S rRNA, ITS1, 5.8S rRNA	End-point	TF211A, TF211B	NA	Does not amplify *Mycoplasma bovigenitalium*, *Ureaplasma diversum* or bovine genomic DNA	1 pg	Reported to produce few unspecific DNA bands	Nickel et al. (2002)
ITS1-5.8S rRNA-ITS2	End-point	Tricho-F/Tricho-R	NA	Amplifies *T. foetus*, *T. suis*, *T. mobilensis* based on in silico analyses; amplified *Pentatrichomonas hominis*	NA	Used in human samples and in a study on cats (Profizi et al., 2013)	Jongwutiwes et al. (2000) and Duboucher et al. (2006)
18S rRNA, ITS1 and 5.8S rRNA	End-point	Forward, reverse	NA	Amplifies *T. foetus* but also trichomonad DNA from a variety of genera; *T. foetus* (157 bp), *Tetratrichomonas* spp. (170–175 bp), *Pentatrichomonas hominis* (142 bp)	Accurate typing is possible from both the 1.0 and 0.1 pg templates	Using diagnostic size variants from within the ITS1 region. Incorporation of a fluorescently labelled primer enables high sensitivity and rapid assessment of results for species identification	Grahn et al. (2005)

Target	Type of PCR	Name of primer	Name of probe (type of probe)	Reported specificity	Reported sensitivity	Remarks	Reference
18S rRNA, ITS1 and 5.8S rRNA	End-point	Forward, reverse 5.8S primer	NA	Amplified *T. foetus* (367 bp), *Tetratrichomonas* sp. (379 bp), *Pentatrichomonas* sp. (333 bp), *T. gallinae* (364 bp), and *T. vaginalis* (363 bp)	0.1 pg	Analysis in a 2% agarose gel and by using fluorescent-labelled primers and 6% polyacrylamide gels; disadvantage: too much template makes typing difficult or impossible; advantage: low costs	Frey et al. (2009)
ITS1/5.8S rRNA/ITS2	End-point nested	TFR3, TFR4 (external); TFITS-F, TFITS-R (internal)	NA	*T. foetus*-specific	1 organism (70% positive), 10 organisms (90% positive), 100 organisms (100% positive); 10 organisms per 200 mg of faeces (90% positive); 100 organisms per 200 mg of faeces (100% positive)	Single tube nested PCR	Gookin et al. (2002)
ITS1/5.8S rRNA/ITS2	Real-time	TFR3, TFR 4	NA	*T. foetus*-specific		SYBR® qPCR	Mueller et al. (2015)
5.8S rRNA	Real-time	T.foeForward (TFF2), T.foeReverse (TFR2)	T.foeProbe (5′FAM/3′MGB-NFQ)	Amplifies *T. foetus*, *T. suis*, *T. mobilensis*	3 fg DNA, 0.1-1 cells per assay	5′ Taq nuclease assay using a 3′ minor groove binder-DNA probe; no need for post-amplification processing	McMillen and Lew (2006)
SSU rDNA	End-point nested	External: 16Sl; 16Sr; Internal: TN3, TN4	NA	Amplifies *Trichomonas* sp.	NA	NA	Robinson et al. (2010)

Target	Type of PCR	Name of primer	Name of probe (type of probe)	Reported specificity	Reported sensitivity	Remarks	Reference
SSU rDNA	End-point	Tgf, Tgr	NA	Amplifies *T. gallinarum*	1 protozoon per assay	Cross-reactions with *T. gallinae*. No cross-reactions were observed with samples from other protozoa (*Toxoplasma gondii*, *Eimeria tenella*, *Cryptosporidium* spp., *E. invadens* and *E. ranarum*)	Grabensteiner and Hess (2006)
Not reported	End-point + southernblot by probe	TF1, TF2	Probe for Southern blot	Amplifies *T. foetus*	10 or occasionally fewer protozoa	Southern blot necessary to identify specific band. A 400 bp product from bovine genomic DNA is amplified. Multiple amplification products from DNA from a related organism, *T. vaginalis*; Southern blot is negative for *T. vaginalis*)	Ho et al. (1994)

Annex A – Mapped fact-sheet used in the individual judgement on trichomonosis

Annex A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2017.4992