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Short communication

Tritrichomonas foetus: Prevalence study in naturally mating bulls in Switzerland

Ch. Bernasconi[a], M. Bodmer[b], M.G. Doherr[c], F. Janett[d], A. Thomann[e], C. Spycher[a], C. Iten[f], B. Hentrich[a], B. Gottstein[a], N. Müller[a], C.F. Frey[a,*]

[a] *Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[b] *Herd Health Service, Clinic for Ruminants, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[c] *Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[d] *Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland*
[e] *Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[f] *Swissgenetics, Hauptstrasse 61, 5243 Mülligen, Switzerland*

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ABSTRACT

Switzerland is officially free from bovine *Tritrichomonas foetus*. While bulls used for artificial insemination (AI) are routinely examined for this pathogen, bulls engaged in natural mating, as well as aborted fetuses, are only very sporadically investigated, indicating that the disease awareness for bovine tritrichomoniasis is low. Natural mating in cattle is becoming increasingly popular in Switzerland. Accordingly, a re-introduction/re-occurrence of *T. foetus* in cattle seems possible either via resurgence from a yet unknown bovine reservoir, or *via* importation of infected cattle. The low disease awareness for bovine tritrichomoniasis might favor an unnoticed re-establishment of *T. foetus* in the Swiss cattle population. The aim of our study was thus to search for the parasite, and if found, to assess the prevalence of bovine *T. foetus* in Switzerland. We included (1) bulls over two years of age used in natural mating and sent to slaughter, (2) bulls used for natural service in herds with or without fertility problems and (3) aborted fetuses. Furthermore, the routinely examined bulls used for AI (4) were included in this study. In total, 1362 preputial samples from bulls and 60 abomasal fluid samples of aborted fetuses were analyzed for the presence of *T. foetus* by both *in vitro* cultivation and molecular analyses. The parasite could not be detected in any of the samples, indicating that the maximal prevalence possibly missed was about 0.3% (95% confidence). Interestingly, in preputial samples of three bulls of category 1, apathogenic *Tetratrichomonas* sp. was identified, documenting a proof-of-principle for the methodology used in this study.

1. Introduction

The flagellated bovine protist *Tritrichomonas foetus* (Riedmüller, 1928) is well known as the causative agent of a venereally transmitted disease in cattle that can lead to infertility and abortion (BonDurant, 1997). Apathogenic flagellates like *Tetratrichomonas* sp. may sometimes be detected in preputial samples of bulls and need to be differentiated from *T. foetus* by molecular methods (Campero et al., 2003). In Switzerland and other regions where artificial insemination (AI) flanked by rigorous quarantine testing of the bulls used therein is common, the prevalence of bovine tritrichomoniasis and in parallel the awareness for this disease, is generally low. Indeed, the last reported

* Corresponding author. Tel.: +41 31 631 24 75; fax: +41 31 631 26 22.
E-mail address: caroline.frey@vetsuisse.unibe.ch (C.F. Frey).

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case of a bull infected with *T. foetus* in Switzerland dates from 1997 (Anonymous, 1997). Where natural mating is widely practiced, *e.g.* in Argentina or the U.S.A., bovine tritrichomoniasis is still prevalent (Mardones et al., 2008; Rae et al., 2004; Rodning et al., 2008). In the last decade, beef and dairy herds relying on natural mating have become more and more important in Switzerland. Natural mating is the predominant reproduction type in Swiss beef herds. Accordingly, the number of bulls aged over two years, *i.e.* bulls used for breeding, has increased by about 30% between 2009 and 2012 (Federal Office for Statistics, 2013). In bulls used for natural mating as well as in aborted fetuses testing for *T. foetus* is not mandatory (Anonymous, 1995) and therefore only very sporadically performed. The increasing importance of natural breeding in combination with the current low awareness of bovine tritrichomoniasis might lead to an unnoticed re-establishment of the parasite in Swiss cattle. Very recently, such a re-establishment in an extensively managed beef breed has been reported from Spain (Mendoza-Ibarra et al., 2012). To address these concerns, a study was designed to investigate for the first time in Switzerland the presence of the parasite in a subpopulation of Swiss cattle most likely to be infected: (1) elderly bulls used in natural service, (2) naturally mating bulls from herds with or without fertility problems, and (3) aborted fetuses. Furthermore, the data of the routine examination of bulls used for AI were included in the study.

2. Materials and methods

2.1. Study design and sample size

The sampling period was January to December 2012. We considered male gender, increasing age of bulls, natural mating, fertility problems in the herd, and abortions as risk factors for, or indicators of, infection with *T. foetus* (BonDurant, 1997; Rae et al., 2004; Campero and Gottstein, 2007; Sager et al., 2007; Mendoza-Ibarra et al., 2012). Bulls destined for meat production are usually slaughtered within the first two years of life. As we targeted bulls used for reproduction, we aimed at sampling bulls aged over 24 months. The bull-level test sensitivity was assumed as 0.78 and the specificity as 1.0 (Cobo et al., 2007). Thus, the sample size necessary to obtain a system sensitivity of 99% (with 95% confidence) to detect a prevalence of between 0.001 and 0.005 in a population of approx. 10,000 bulls aged over two years in 2011 in Switzerland ranged from 4732 to 1129 bulls, respectively (http://epitools.ausvet.com.au). Since random sampling of this number of live bulls from the standing population was considered unfeasible, we collaborated with slaughterhouses to target bulls sent for slaughter and available for sampling. In total, 1098 bulls could be sampled by this approach. Additionally, we included samples from 61 live bulls, 14 of these originated from herds with fertility problems such as repeat breeders, embryonic loss, abortions, or increased incidence of chronic clinical endometritis, and also obtained samples from 203 bulls used in AI. This resulted in an effective sample size of 1362 bulls. Additionally, we obtained samples from 60 aborted fetuses.

2.2. Sampling procedure

From bulls sampled at the slaughterhouse, the distal part of the penis including the prepuce and the fornix was collected, sealed in individual plastic bags to avoid loss of moisture, and sent to the Institute of Parasitology in Bern (IPB) using individual plastic containers without cooling. The samples arrived within 24 h at the laboratory. Upon arrival of the samples, the fornix region of the penis/prepuce was scraped with sterile metal scrapers. Part of the collected material was transferred directly to the InPouch™ system (Biomed diagnostics, San José, Ca., USA) and another part was stored at −20 °C for subsequent molecular analyses.

Live bulls used in natural service were sampled by preputial washings using sterile isotonic saline (Schönmann et al., 1994). Those samples were sent in 15 ml Falcon® tubes at ambient temperature and arrived within four to 24 h at the IPB. The sediment was used to inoculate the InPouch™ cultivation system, and for molecular analysis.

Aborted bovine fetuses or abomasal fluid of the fetuses were submitted within 24 h to the IPB without cooling. Upon arrival, fetuses were opened and abomasal fluid was extracted. The abomasal fluid was used to directly inoculate the InPouch™ system, and for molecular analysis.

2.3. In vitro cultivation

All samples were investigated by using the bovine InPouch™ system. The pouches were cultivated in an upright position at 37 °C for three consecutive days (Sager et al., 2007). A positive control pouch was included in each cultivation series. The cultures were controlled for growth of trichomonads by light microscopy (100× magnification) every 24 h. Culture was marked as positive or negative based on the presence of motile flagellated trophozoites. If trophozoites were visible, 100 μl of the culture sediment was used for molecular analysis.

2.4. Purification of genomic DNA and PCRs

Genomic DNA of all samples was extracted following the protocol for cultured cells of the DNeasy® Blood and Tissue Kit (Qiagen, Switzerland). DNA was eluted in 200 μl elution buffer. A panspecific PCR for amplification of the internal transcribed spacer region 1 (ITS1) of various trichomonads was performed as earlier described (Frey et al., 2009). Forward 18S primer (5′-GTAGGTGAACCTGCCGTTG-3′) and reverse 5.8S primer (5′-TTCAGTTCAGCGGGTCTTC-3′) (MWG-Biotech Inc., Germany) were used. For *T. foetus*, amplification products of 367 bp were anticipated, and for *Tetratrichomonas* sp. amplification products of 370 to 400 bp were anticipated. To prevent carry-over contamination from previous diagnostic reactions, uracyl DNA glycosylase (UDG) and dUTP (instead of dTTP) were included in the reaction mixture (Longo et al., 1990). Briefly, one PCR (total volume 25 μl) consisted of 2 μl DNA, 2.5 μl 10× PCR buffer (Perkin-Elmer, Rotkreuz, Switzerland), 0.2 mM each dATP, dGTP, and dCTP, 0.4 mM dUTP (Amersham Biosciences), 6.25 pmol each of the primers, 2 units

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of AmpliTaq™ DNA polymerase (Perkin-Elmer) and 0.5 units of uracil DNA glycosylase (UDG; Roche, Switzerland). $MgCl_2$ was supplemented to a final concentration of 3.5 mM.

Amplification products were visualized by agarose gel electrophoresis (2%) using ethidiumbromide staining. A PCR specific for *Tritrichomonas* sp. (Felleisen et al., 1998) using forward primer TFR4 (5′-CCTGCCGTTGGATCAGTTT CGTTAA-3′) and reverse primer TFR3 (5′-CGGGTCTTCC TATATGAGACAGAACC-3′) was additionally performed with samples showing a positive *in vitro* culture and positive panspecific amplification product. One reaction of 25 μl consisted of 2 μl DNA, 2.5 μl 10× PCR buffer (Perkin-Elmer), 0.2 mM each dATP, dGTP, and dCTP, 0.4 mM dUTP (Amersham Biosciences), 6.25 pmol each of the primers, 2 units of AmpliTaq™ DNA polymerase (Perkin-Elmer) and 0.5 units of UDG (Roche). $MgCl_2$ was supplemented to a final concentration of 2.5 mM.

2.5. Cloning and sequencing

Of all samples of which flagellates were grown *in vitro*, the ITS1 was amplified from genomic DNA with the high fidelity, proof-reading DNA polymerase phusion (New England BioLabs M0530L) using the primers of the panspecific PCR (forward 5′-GTAGGTGAACCTGCCGTTG-3′ and reverse 5′-TTCAGTTCAGCGGGTCTTC-3′). The PCR product was ligated into pCR-Blunt II TOPO vector (Invitrogen 45-0245) according to manufacturer's protocol, transformed into chemo-competent *Escherichia coli* (Top10, Invitrogen) and grown under Kanamycin selection. Plasmid DNA of positive clones was extracted using a QIAprep spin miniprep kit (QIAGEN cat no. 27106) according to manufacturer's protocol. For dye incorporation, a sequencing reaction using BigDye Terminator (Applied Biosystems 4336699) was used according to manufacturer's protocol. DNA was ethanol precipitated and taken up in Hi-Di Formamide (Applied Biosystems 4311320). Samples were run on an ABI3730 sequence analyzer. Sequences were analyzed using following online tools: Basic Local Alignment Search Tool nBLAST from NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome), Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/), and reverse-complement.com.

2.6. Characterization of sampled bulls

All sampled bulls were identified in the animal data base (Identitas AG, Bern, Switzerland). Origin, age, and breed were recorded and compared with the according data of all bulls older than two years living in 2012 in Switzerland.

2.7. Statistical analyses

For statistical analyses, only the data from bulls aged over two years were included. The target population was all bulls aged over 24 months that lived or were slaughtered in 2012 in Switzerland. The probability of freedom achieved for *T. foetus* in Swiss bulls was calculated using the "Population Level Sensitivity module" (epitools.ausvet.com.au) with the following inputs: Sample size = 1194, population size = 12,445, sensitivity of test method (culture and PCR) = 0.783 (Cobo et al., 2007), specificity of test method (culture, PCR, and sequencing) = 1.0, a desired confidence of 0.95 and design prevalences of 0.002, 0.003 and 0.004.

Table 1
Origin, age, breed and activity of sampled bulls compared to the respective values in the target population.

	Analyzed bulls* (n = 1194)	Target population** (n = 12,445)
	n (%)	n (%)
Origin		
Switzerland	1163 (97.5)	12,159 (97.7)
France	18 (1.5)	100 (0.8)
Other Europe	13 (1.1)	187 (1.5)
Age		
2–3 years	334 (28)	6011 (48.3)
3–4 years	423 (35.4)	3708 (29.8)
4–5 years	191 (16)	1195 (9.6)
5–6 years	111 (9.3)	585 (4.7)
6–7 years	60 (5)	373 (3)
7–8 years	39 (3.3)	249 (2)
8 years or older	36 (3)	336 (2.7)
Breed		
Red Holstein	254 (21.3)	1568 (12.6)
Limousin	187 (15.7)	2029 (16.3)
Holstein Friesian	169 (14.2)	871 (7)
Brown Swiss	156 (13.1)	1.605 (12.9)
Simmental	147 (12.3)	722 (5.8)
Other	281 (23.4)	5650 (45.4)
Activity		
Natural mating	1123 (94.1)	Na
AI	71 (5.9)	Na

na: Data not available.
* Sampled bulls aged over 24 months.
** All bulls aged over 24 months living in Switzerland in 2012.

3. Results

3.1. Description of sampled bulls

We obtained the information about the country of origin for all bulls included in our study (n = 1362; 100%). The birth date and breed was recorded for 1357 bulls (99.6%). 163 bulls (11.9%) were younger than two years at the time of sampling. Of these, 134 were bulls used in AI and 23 were naturally mating bulls sampled in their herds. Six bulls sampled at the slaughterhouse were younger than two years. Origin, age, and breed of the bulls aged over two years in our sample (n = 1194) were compared with the respective values in the target population (all bulls aged over two years living in 2012 in Switzerland; n = 12,445) (Table 1). The composition of our sampled bulls mirrored very nicely the target population in terms of origin of the animals, as well as for the age distribution, although in our sample the bulls aged over three years were slightly overrepresented (Table 1). In terms of production types, a slight overrepresentation of breeds used in milk production (Red Holstein, Holstein Friesian), or two purpose breeds (Simmental) in the sampled population was observed.

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3.2. Detection of *T. foetus* and *Tetratrichomonas* sp.

Scrapings from 1098 bulls' penises, preputial washings from 61 naturally mating bulls, and 203 AI bulls, respectively, and abomasal fluids from 60 aborted fetuses were tested in parallel by *in vitro* culture and PCR. Samples from preputial washings and abomasal fluids were all negative by both methods. In the cultures of three scrapings from slaughtered bulls, motile trophozoites could be observed during all three days ($n = 2$) or at one day ($n = 1$), respectively, (Table 2) of cultivation. Subculturing in new InPouch^{TM} cultures and in Diamonds medium (Diamond, 1957) failed. Panspecific PCR (Frey et al., 2009) of these three samples yielded amplification products of 378, 393 and 399 bp, respectively, which were larger than the 367 bp product expected for *T. foetus*. Subsequent cloning of the amplification products and DNA sequencing yielded *Tetratrichomonas* sp. for all samples. The closest matches in GenBank as well as additional information on the bulls are shown in Table 2. Furthermore, PCR specific for *Tritrichomonas* sp. (Felleisen et al., 1998) was performed for all three samples and remained negative. Based on these results, we concluded that all samples tested were negative for *T. foetus*. As in our study the system sensitivity to detect a prevalence of 0.002, 0.003 and 0.004 was 0.893, 0.967 and 0.990, respectively, we could conclude that the maximum prevalence that could have been missed was 0.3% (with 95% confidence).

4. Discussion

Natural mating is becoming increasingly popular in the Swiss cattle husbandry, but bulls used in this system are not routinely tested for venereal diseases. A re-introduction/re-occurrence of bovine *T. foetus* is conceivable either *via* resurgence from a yet unknown bovine reservoir (Mendoza-Ibarra et al., 2012), or *via* importation of infected cattle. The low disease awareness for bovine tritrichomoniasis might favor a hidden re-occurrence of *T. foetus* in the Swiss cattle population. The aim of our study was thus to search for the parasite, and if found, to assess the prevalence of bovine *T. foetus* in Switzerland.

In the present epidemiological survey, which is the first of this kind for Switzerland, we applied two sampling methods on bulls, namely preputial scrapings (from dead bulls) and preputial washings (from living bulls). To avoid death of the fragile trichomonads during their transport to the IPB, precautions were taken to avoid desiccation or thermal damage of the samples by sealing the penises/prepuces in plastic bags, and by shipping all samples at room temperature. However, to avoid false negative results in case of parasite death during transport, we did not solely rely on culture but performed PCR in parallel on each sample. The combination of culture and PCR on one sample had been shown to result in a diagnostic sensitivity of 78.3% (Cobo et al., 2007). Sensitivity could be optimized by repeated sampling respective to individual bulls (Cobo et al., 2007). In our study, maximal diagnostic sensitivity could not be achieved because most of our bulls could only be sampled once, namely upon slaughter. However, our sampling strategy had several advantages

Table 2
Characterization of the three bulls positive for *Tetratrichomonas* sp.

Bull no.	Breed	Age in years	Origin	Sampled at	Length of amplicon[1]	GenBank comparison (% identity; % coverage; *e*-value)	Species	Reference
641	Limousin	4	CH	SH	393 bp	AY886838.1 (99%; 99%; <2*e* − 172)	*Tetratrichomonas* sp.	Cepicka et al. (2006)
816	Angus	4	CH	SH	378 bp	AF342742.1 (96%; 100%; 1*e* − 164)	*Tetratrichomonas* sp.	Walker et al. (2003)
847	Red Holstein	1.5	CH	SH	399 bp	AY886838.1 (98%; 98%; 2*e* − 172)	*Tetratrichomonas* sp.	Cepicka et al. (2006)

CH: Switzerland; SH: slaughterhouse.
[1] Reference for PCR: Frey et al. (2009).

that prompted us to tolerate this limitation: (1) the labor-intensive and potentially dangerous task of immobilizing the bull to take a preputial washing or scraping was not necessary, (2) a large number of bulls could be sampled within just one year, and (3) all post-mortem samples were processed by the same person under standardized laboratory conditions, thus reducing variations in diagnostic sensitivity due to changing handling of the samples. The detection of three bulls that harbored apathogenic *Tetratrichomonas* sp. indirectly confirmed the appropriateness of our methodical approach to diagnose and identify/specify trichomonad infections. These three samples were taken from dead bulls and both, culture and initial PCR, were positive for trichomonads. Previous reports reported findings of non-*T. foetus* trichomonads in preputial samples of routinely examined bulls and suggested that *Tetratrichomonas* sp. most likely represented contaminations from the digestive tract (Taylor et al., 1994; Cobo et al., 2003; Campero et al., 2003; Walker et al., 2003; Dufernez et al., 2007; Corbeil et al., 2008; Huby-Chilton et al., 2009).

The present survey unveiled two practices that seem to be widely used in herds using natural mating in Switzerland and that are both beneficial for the prevention of *T. foetus* infections: (1) the dominant use of young bulls in natural mating (Campero and Gottstein, 2007) as reflected by the age curve of both our sampled and the target population, and (2) the almost exclusive use of indigenous bulls, thus reducing the risk of importation of the disease. Furthermore, our study revealed that whole fetuses are only sporadically submitted for laboratory analyses. Thus, we could only test a limited number of abomasal samples that was insufficient to fulfill the statistical requirements of the present type of epidemiological survey.

Some concerns for cattle health had arisen after the detection of widespread infection of cats with feline *T. foetus* (Burgener et al., 2009; Frey et al., 2009), as cats on farms normally have unlimited access to barns and cattle, and as experimental transmission from cats to cattle is possible (Stockdale et al., 2007). However, recent studies unambiguously demonstrated that feline and bovine *T. foetus* exhibit conserved genetic differences (Reinmann et al., 2012; Slapeta et al., 2010, 2012; Sun et al., 2012), thus suggesting different host tropism (Slapeta et al., 2012).

5. Conclusions

Based on the results of this study, bulls used for natural mating in Switzerland have a probability of less than 0.3% of being infected with bovine *T. foetus*. However, we cannot exclude that the parasite might be present in some breeds and under particular forms of management, or that it might be re-introduced *via* infected imported animals. Therefore, despite of Switzerland being free of bovine tritrichomoniasis, disease awareness should be maintained in the future, and a statutory testing regimen for bulls imported from endemic areas should be considered.

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References

Anonymous, 1995. Tierseuchenverordnung No 916.401, http://www.admin.ch/opc/de/classified-compilation/19950206/index.html.
Anonymous, 1997. Tierseuchenstatistik, https://www.infosm.bvet.admin.ch/public/awzeit/auswertung/.
BonDurant, R.H., 1997. Pathogenesis, diagnosis, and management of trichomoniasis in cattle. Vet. Clin. North Am. Food Anim. Pract. 13, 345–361.
Burgener, I., Frey, C., Kook, P., Gottstein, B., 2009. *Tritrichomonas foetus*: a new intestinal parasite in Swiss cats (in German). Schweiz. Arch. Tierheilkd. 151, 383–389.
Campero, C.M., Rodriguez Dubra, C., Bolondi, A., Cacciato, C., Cobo, E., Perez, S., Odeon, A., Cipolla, A., BonDurant, R.H., 2003. Two-step (culture and PCR) diagnostic approach for differentiation of non-*T. foetus* trichomonads from genitalia of virgin beef bulls in Argentina. Vet. Parasitol. 112, 167–175.
Campero, C.M., Gottstein, B., 2007. Tritrichomoniasis: control measures. In: Ortega-Mora, L.M., Gottstein, B., Conraths, F.J., Buxton, D. (Eds.), Protozoal Abortion in Farm Ruminants: Guidelines for Diagnosis and Control. CAB International, Wallingford, pp. 290–301.
Cepicka, I., Hampl, V., Kulda, J., Flegr, J., 2006. New evolutionary lineages, unexpected diversity, and host specificity in the parabasalid genus *Tetratrichomonas*. Mol. Phylogenet. Evol. 39, 542–551.
Cobo, E.R., Campero, C.M., Mariante, R.M., Benchimol, M., 2003. Ultrastructural study of a tetratrichomonad species isolated from preputial smegma of virgin bulls. Vet. Parasitol. 117, 195–211.
Cobo, E.R., Favetto, P.H., Lane, V.M., Friend, A., VanHooser, K., Mitchell, J., BonDurant, R.H., 2007. Sensitivity and specificity of culture and PCR of smegma samples of bulls experimentally infected with *Tritrichomonas foetus*. Theriogenology 68, 853–860.
Corbeil, L.B., Campero, C.M., Van Hoosear, K., Bondurant, R.H., 2008. Detection of trichomonad species in the reproductive tracts of breeding and virgin bulls. Vet. Parasitol. 154, 226–232.
Diamond, L.S., 1957. The establishment of various trichomonads of animals and man in axenic cultures. J. Parasitol. 43, 488–490.
Dufernez, F., Walker, R.L., Noël, C., Caby, S., Mantini, C., Delgado-Viscogliosi, P., Ohkuma, M., Kudo, T., Capron, M., Pierce, R.J., Villanueva, M.R., Viscogliosi, E., 2007. Morphological and molecular identification of non-*Tritrichomonas foetus* trichomonad protozoa from the bovine preputial cavity. J. Eukaryot. Microbiol. 54, 161–168.
Federal Office for Statistics, 2013. Landwirtschaftliche Betriebsstrukturerhebung 2009–2012. Federal Office for Statistics, www.bfs.admin.ch.

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

Frey, C.F., Schild, M., Hemphill, A., Stünzi, P., Müller, N., Gottstein, B., Burgener, I.A., 2009. Intestinal *Tritrichomonas foetus* infection in cats in Switzerland detected by *in vitro* cultivation and PCR. Parasitol. Res. 104, 783–788.

Huby-Chilton, F., Scandrett, B.W., Chilton, N.B., Gajadhar, A.A., 2009. Detection and identification of *Tetratrichomonas* in a preputial wash from a bull by PCR and SSCP. Vet. Parasitol. 166, 199–204.

Longo, M.C., Berninger, M.S., Hartley, J.L., 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene 93, 125–128.

Mardones, F.O., Perez, A.M., Martínez, A., Carpenter, T.E., 2008. Risk factors associated with *Tritrichomonas foetus* infection in beef herds in the Province of Buenos Aires. Argent. Vet. Parasitol. 153, 231–237.

Mendoza-Ibarra, J.A., Pedraza-Díaz, S., García-Peña, F.J., Rojo-Montejo, S., Ruiz-Santa-Quiteria, J.A., San Miguel-Ibáñez, E., Navarro-Lozano, V., Ortega-Mora, L.M., Osoro, K., Collantes-Fernandez, E., 2012. High prevalence of *Tritrichomonas foetus* infection in Asturiana de la Montaña beef cattle kept in extensive conditions in Northern Spain. Vet. J. 193, 146–151.

Rae, D.O., Crews, J.E., Greiner, E.C., Donovan, G.A., 2004. Epidemiology of *Tritrichomonas foetus* in beef bull populations in Florida. Theriogenology 61, 605–618.

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

Riedmüller, L., 1928. Uebertragungsversuche und klinische Bedeutung der beim sporadischen Abortus des Rindes vorkommenden Trichomonaden (Zentralbl. Bakteriol. I. Abt. Orig.). In: Ueber die Morphol., pp. 103–118.

Rodning, S.P., Wolfe, D.F., Carson, R.L., Wright, J.C., Stockdale, H.D., Pacoli, M.E., Busby, H.C., Rowe, S.E., 2008. Prevalence of *Tritrichomonas foetus* in several subpopulations of Alabama beef bulls. Theriogenology 69, 212–217.

Sager, H., Ferre, I., Henning, K., Ortega-Mora, L., 2007. Tritrichomoniasis: aetiological diagnosis. In: Ortega-Mora, L.M., Gottstein, B., Conraths, F.J., Buxton, D. (Eds.), Protozoal Abortion in Farm Ruminants: Guidelines for Diagnosis and Control. CAB International, Wallingford, pp. 232–262.

Schönmann, M.J., BonDurant, R.H., Gardner, I.A., Van Hoosear, K., Baltzer, W., Kachulis, C., 1994. Comparison of sampling and culture methods for the diagnosis of *Tritrichomonas foetus* infection in bulls. Vet. Rec. 134, 620–622.

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

Slapeta, J., Müller, N., Stack, C.M., Walker, G., Lew-Tabor, A., Tachezy, J., Frey, C.F., 2012. Comparative analysis of *Tritrichomonas foetus* (Riedmüller, 1928) cat genotype, *T. foetus* (Riedmüller, 1928) cattle genotype and *Tritrichomonas suis* (Davaine, 1875) at 10 DNA loci. Int. J. Parasitol. 42, 1143–1149.

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

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

Taylor, M.A., Marshall, R.N., Stack, M., 1994. Morphological differentiation of *Tritrichomonas foetus* from other protozoa of the bovine reproductive tract. Br. Vet. J. 150, 73–80.

Walker, R.L., Hayes, D.C., Sawyer, S.J., Nordhausen, R.W., Van Hoosear, K.A., BonDurant, R.H., 2003. Comparison of the 5.8S rRNA gene and internal transcribed spacer regions of trichomonadid protozoa recovered from the bovine preputial cavity. J. Vet. Diagn. Invest. 15, 14–20.

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