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REVIEW ARTICLE

Tritrichomonas foetus as a causative agent of tritrichomonosis in different animal hosts

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Abstract

Tritrichomonas foetus is a protozoan parasite that has been traditionally identified as a cause of reproductive tract disease in cattle and gastrointestinal tract infection in cats. Moreover, *T. foetus* is also well known as a commensal of the nasal cavity, intestines, and stomach in swine. In this review we describe *T. foetus* as a pathogen dangerous to more than one animal host, diagnostic and taxonomic aspects of this infection, and the extent to which isolates from different hosts share genetic identity.

Keywords: animals, *Tritrichomonas foetus*, tritrichomonosis, diagnostics.

Introduction

Tritrichomonas foetus is a very intriguing protozoan parasite which can occur in different host species. It is an obligate parasite of the bovine reproductive and feline gastrointestinal tracts and may lead to a disease called tritrichomonosis. *T. foetus* is also a commensal of pigs and can be found in their nasal cavities, intestines, and stomachs. Identification studies on this dangerous parasite have been conducted for many decades and nowadays the level of advancement of diagnostic methods allows for very accurate identification of *T. foetus*. There is still much speculation on *T. foetus*' origin, identity, and taxonomy, and the question remains open whether, when isolated from different hosts, the parasite belongs to the same species. Here, we review the pathogenesis of tritrichomonosis in animal hosts and methods of the identification of *T. foetus*. We also give an overview of its biology and aetiology, as well as of the studies on the identity and genetic diversity of bovine, feline, and porcine isolates.

Causative agent

Tritrichomonas foetus was observed and isolated from the bovine vagina and porcine intestine by Kunstler in 1888 and Mazzanti in 1990, respectively. After 28 years Riedmüller gave the name of *Trichomonas foetus* to this parasite infecting cattle all over the world. The name of the parasite was finally established as *Tritrichomonas foetus* by Wenrich and Emmerson based on analysis of its morphology (35).

Tritrichomonas foetus is a single-cell parasite belonging to the Trichomonadidae family, Trichomonadorida order, Sarcomastigophora phylum. The pear- or spindle-shaped body of *T. foetus* is 10–20 μm long and 5–15 μm wide with three anterior flagella and a posterior flagellum. The fourth flagellum runs towards the undulating membrane around ¾ of the body, forming its edge and is free beyond the back of the body.

In the front part of the *T. foetus* cell, a large single nucleus and uncommon structures such as a parabasal apparatus are visible. *Tritrichomonas foetus* has no mitochondria and instead contains spherical hydrogenosomes which regulate the metabolism of parasite cells. Just as all flagellates have, *T. foetus* possesses a well-developed cytoskeleton composed of an axostyle, costa, and pelta. The most characteristic part is the axostyle, which is thick, contains granules in the capitulum and from the posterior end of which a chromatic ring emerges. The costa is made of striated fibres and runs just below the surface of the cell membrane at the basis of the undulating membrane. The pelta is a moon-shaped

microtubular accessory organ for the flagella which also supports the wall of the periflagellar canal.

Tritrichomonas foetus resides in *reproductive tract* tissues. When growing on medium cultures, it most often adopts the spindle-shaped form called a trophozoite. The biology and structure of this form are the most common and well known. However, in unfavourable circumstances such as a lack of nutrients, the presence of drugs or decreasing temperature, trichomonad cells can transform into a stage called a pseudocyst. Pseudocysts have a spherical shape without flagellates or an undulating membrane. For several years it was thought that pseudocysts were the degradative form of *T. foetus* and knowledge about their role in the life cycle of the parasite was lacking. Nowadays, analyses reveal that most of the parasites adopt the pseudocyst form in fresh preputial and swab samples. Far from being degradative, they are a defence mechanism which protects trichomonad cells from poor environmental conditions.

Bovine tritrichomonosis

Tritrichomonas foetus in cows is found on the mucosal surfaces of the uterus and vagina. The characteristic symptoms of this disease are chronic inflammation of the reproductive tract and reproductive failure. After inoculation of the parasite into the genital tract, infection occurs in the vagina, cervix, endometrium, and tubules. Infected cows suffer from vaginitis, cervicitis, endometritis, and infertility. A minor percentage of infected cows develop pyometra and it can be a major indicator of tritrichomonosis. Trichomonad infection can cause early embryonic mortality and, less often, abortion. Infertility in cows is thought to be a consequence of early embryonic death. Inflammation of the mucosal surfaces of the reproductive tract causes placental oedema, mild lymphocytic and histiocytic chorionitis, and focal necrosis of trophoblasts. In half of bovine tritrichomonosis cases foetal pneumonia with intrabronchiolar neutrophils can occur, and macrophages, and multinucleated giant cells can be observed (49).

In contrast, bulls remain healthy, but shortly after infection preputial discharge associated with small nodules on penile membranes may occur. Despite this possibility, infected bulls are usually asymptomatic carriers; they are important agents for distributing disease because a small number of trichomonads in the preputium, fornix, and around the glans penis can be transmitted during fertilisation. Infection of the cow is caused by sexual contact with an infected bull. In the first stage of infection cows do not have rapid conception failure. The pregnancy progresses up to 120 days although the abortion typically occurs around the 70–90th day, during which time the embryo/foetus dies and is resorbed or expelled. Cows with tritrichomonosis usually clear the infection within a few months, entailing a longer breeding season in affected herds.

Tritrichomonosis is a widespread disease reported for the first time in 1932 in Pennsylvanian cattle (13). The prevalence of infection in cattle is not the same in all regions of the world. In countries where artificial insemination is used on a large scale as a main method of cattle reproduction, the possibility of trichomonads spreading is significantly limited. Therefore, the prevalence of *T. foetus* in these regions has decreased almost to zero. For example, in the north-west of the USA, the prevalence of *T. foetus* is very low and in North Carolina, no carriers were detected in a 1995 study (15). However, in areas where cattle breeding is carried out extensively and animals are naturally reproduced, tritrichomonosis may spread among a large percentage of animals. In the USA tritrichomonosis has been endemic since the 1980's and cases of the diseases have been noted in many states: especially the Midwest and western states including California, Alabama, Colorado, Idaho, Missouri, New Mexico, Nebraska, Nevada, and Oklahoma. Moreover, *T. foetus* was identified also in Kansas, South Dakota, Utah, and Wyoming (61). The highest percentage of positive results in USA was noted in Florida where infection with *T. foetus* was reported in 10% of medium dairy cattle herds (100–499 animals) and 53% of large cattle herds (≥ 500 animals) (43).

A similar epidemiological situation was observed in certain countries of South America, South Africa, and Asia, and in Australia. The prevalence of tritrichomonosis in Argentina was 3.5% (30) and was 3.7% in Brazil (12) where natural service was the main way of reproduction. A study conducted in Costa Rica showed that 18.4% of cows and 7.2% of bulls had *T. foetus* infection (42). The percentage of infected bulls in Africa varied, being 10.4% in the north-western part (10) while in Transkei, South Africa, protozoan parasites were detected in 23 (26.4%) of 87 examined animals.

Aborted foetuses totalling 45 (56.3%) out of 80 in 12 dairy herds in Beijing, China, were positive (60). Additionally, bovine tritrichomonosis occurred in Rajasthan in India with 28.16% (29/103) (41) and in Turkey (22) with 5.7% prevalence. Studies conducted in Australia revealed that 65.9% of examined bulls were positive in the Victoria River district between 1985 and 1986 (32).

Most European countries are free of tritrichomonosis because of the use of artificial insemination as a main way of reproduction and the implementation of control programmes. However, the disease is still being diagnosed. For example in Spain *T. foetus* was detected in 32% of tested bulls in 2011 (34) and the World Organisation for Animal Health reported the presence of this bovine disease in Portugal and France in 2016. Additionally, tritrichomonosis occurred in Malta and Moldova in 2001 (58).

Feline tritrichomonosis

During the last few decades *T. foetus* has been detected among cats, where it is an obligate parasite

living in the gastrointestinal tract. *T. foetus* is reported as a pathogen of the large intestine of cats and colonises the ileum, caecum, and colon.

The first symptoms of feline tritrichomonosis can occur within 2–9 days after inoculation of the parasite. Firstly, just after infection, trichomonads are frequently observed in contact with surface mucus or adhering to enterocytes along the surface epithelium and crypts. *T. foetus* cells have cytotoxic and proteolytic activity and this ability plays the main role in tissue infection and host cell destruction. The other steps by which *T. foetus* achieves host pathogenicity are interaction and adhesion of the parasite cells to mucus and subsequently to the mucosal epithelium. Histological studies revealed that generally trichomonads are present in close proximity to the mucosal surface and less frequently in the lumen of colonic crypts, and infection can cause neutrophilic colitis, crypt microabscesses, and attenuation of the colonic mucosa. The presence of colonic trichomonads was consistently associated with mild-to-moderate lymphoplasmacytic and neutrophilic colitis, crypt epithelial cell hypertrophy, hyperplasia and increased mitotic activity, loss of goblet cells, crypt microabscesses, and attenuation of the superficial colonic mucosa (59). Feline tritrichomonosis leads to several symptoms which may include chronic diarrhoea associated with blood and mucus. The diarrhoea can be firm or loose in consistency. Cats also suffer from tenesmus, flatulence, and anal irritation (52).

Often affected animals maintain relatively good condition, but mostly typical symptoms like depression, anorexia, hyporexia, vomiting, and weight loss have been noted. Tritrichomonosis in cats has an ambiguous course and manifests with signs varying from barely perceptible (in subclinical carriage) to intractable, chronic large-bowel diarrhoea, and frequently includes periods without evident clinical symptoms (19).

T. foetus is transmitted by the faecal-oral route and trichomonads are able to survive in moist environments for several days outside the host. Therefore, shared litter boxes and mutual grooming can be potential sources of feline infection. Moreover, according to Rosypal *et al.* (47) *T. foetus* can survive and be potentially infectious in dry and canned cat feed as well as in water up to 24 h.

The disease occurs in populations of animals living in close contact and in high density, and therefore it is mostly catteries and shelters which are the most important risk factor for feline tritrichomonosis. Cats suffering from tritrichomonosis can also be feral as well as in shelters (26). The survey conducted by Stockdale *et al.* (52) showed that there is no significant correlation between sex and breed but animals under one year old are definitely more susceptible. Older affected cats are more likely asymptomatic or have a history of diarrhoea during kittenhood.

The first case of tritrichomonosis in cats was identified in 1996 (20) and since that time the disease has occurred worldwide as one of the most common reasons for gastrointestinal tract disorders in cats. Tritrichomonosis occurred in Australia with a prevalence of 42.4% among young cats (1) and in New Zealand with 81.8% among pedigree animals from 12 catteries (26).

Studies conducted in Canada show that the highest amount of positive cases (23.6%) were identified in animals from cat shows (24). Several surveys were also carried out in the USA and in 26 cats with diarrhoea, 22 animals were found to have tritrichomonosis (14). Additionally, among 152 tested animals the disease occurred in 32 diarrhoeic animals (19). Moreover, studies conducted by Gookin *et al.* (21) gave 31% positive results among 117 cats tested during cat shows in the USA. Investigations carried out by Stockdale *et al.* (52) on a large scale with feline faecal samples obtained from US state veterinarians indicated that there was no correlation between sex or breed and predilection for the disease, and tritrichomonosis occurred among young cats with a prevalence of 9.8%. *T. foetus* was also detected in a feline population in Asia. The prevalence of the parasite in Japanese cats was 8.8% (8) among animals in three different hospitals. Studies carried out in China between 2009 and 2014 showed that *T. foetus* was more often found in young purebred cats (86%) (28).

The disease has been noted in many countries in Europe: Germany, Italy, Switzerland, Greece, France, Poland, the UK, Austria, Finland, the Netherlands, Norway, Spain, and Sweden. The prevalence of *T. foetus* infection was in the range of 2–30% among the European cat population (5, 62).

T. foetus as a commensal in pigs

T. foetus was identified in pigs and is also widely known under the synonym *T. suis* because it is considered to be a commensal in suidae. It commonly colonises the nasal cavity, stomach, caecum, and colon, and is occasionally found in the small intestine.

Examining for and reporting tritrichomonosis in pigs is not mandatory and because of this the real prevalence of infection is not known. However, studies have revealed that the prevalence of *T. foetus* (*T. suis*) among the pig population is relatively high. According to Hibler *et al.* (23), the parasite was found in the nasal cavity of 55% of animals tested, in the stomach of 8%, the small intestine of 3%, and the caecum of 43%. In Japan, molecular studies of faecal samples from pigs showed a prevalence of 56.3% (7) and a survey conducted in China elicited that the infection rates of *T. foetus* in the gastrointestinal tract of pigs exceeds 12% (29). Other surveillance reported positive results for the presence of the parasite in 65% of Australian pigs in a mixed farming system with *T. foetus*-negative cattle (37). Most cases of *T. foetus* in pigs are described as devoid of apparent clinical signs; however, the parasite was identified as the causative agent of pulmonary tritrichomonosis in a 40-day-old piglet (48).

It should be noted that high prevalence of *T. foetus* in pigs may increase the risk of its transmission to cattle and cats.

Diagnostic methods

Tritrichomonosis among animal hosts manifests in different ways and it is difficult to attribute a disorder to the parasite only based on clinical signs. Therefore, guidelines contained in the OIE Terrestrial Manual list methods for *T. foetus* identification. The material for bovine *T. foetus* identification is collected from the preputial cavity (liquid), the vagina (mucus) or aborted foetuses. Specimens for studies from cats are rectal swabs and faecal samples. In the case of pigs, swabs from the nasal cavity and faeces are tested.

Microscopy

The most common and widely used method of *T. foetus* detection is direct microscopic examination, which should be made immediately after sample collection and performed under a light microscope with 100–400 × magnification.

A positive result is defined by the presence of trichomonad cells under the microscope as live motile organisms, and a negative result is lack of *T. foetus.* The sensitivity of microscopic examination is estimated to be from 38% to 82% and depends on the number of cells presented in the sample. When specimens cannot be tested immediately after collection it is necessary to use transport medium or culture medium with antibiotics. The medium contains different substances like serum, carbohydrates, egg yolk, or egg white for promoting cell growth.

Tritrichomonads grow rapidly on culture medium and methods exploiting this characteristic are considered the gold standard for diagnosis of the disease. Bacterial contamination of cell culture can be avoided by periodically sieving parasites into new culture medium. The most common and universal culture medium for trichomonads is Diamond medium which has been widely used for 60 years with only minor modifications (3). After inoculation with the parasite, a cell culture is incubated at 37°C and should be examined for *T. foetus* growth daily for up to five days. A drop of the medium from the lower portion of the tube should be examined by wet mount for the presence of motile trophozoites. If no trichomonad cells are observed after five days then the test is considered negative. Recently, the InPouch medium has become more common and it can also be a transport medium, having an optimal incubation temperature of 25–37°C. The medium includes components inhibiting bacterial and fungal growth, which is very important, especially for faecal samples from cats.

It must be noted that *T. foetus* is extremely difficult to distinguish from other flagellates based on its morphology alone. Because several microorganisms similar to *T. foetus* (in size, shape, and motility) may be found in samples from cattle, routine microscopic examination can give false positive results. For example, in the study conducted by Dufernez *et al.* (9), twelve non-*T. foetus* flagellates were identified in samples from the preputial cavity of bulls (*Tetratrichomonas* sp., *Pentatrichomonas hominis, Pseudotrichomonas*, or *Monocercomonas*) – which can be mistaken for *T. foetus* in routine tests.

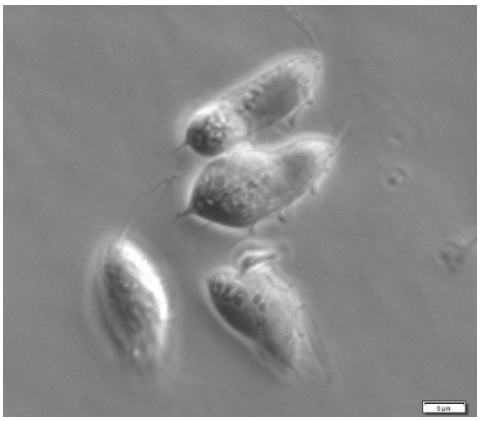

Fig. 1. *Tritrichomonas foetus* trophozoites observed under a light microscope (100×, differential interference contrast)

Molecular methods

The microscopic methods being limited in utility, some molecular techniques to identify *T. foetus* have been elaborated.

Polymerase chain reaction (PCR) allows efficient detection of DNA even from dead parasites or when the number of trichomonads is low. This technique is used successfully both in combination with culture or alone. There are many varieties of molecular methods for tritrichomonosis detection. Genetic studies of bovine tritrichomonosis were mainly carried out within ribosomal RNA genes, especially ITS-1, 5.8S, and ITS-2. TFITS-F/ TFITS-R, TFR1 / TFR2, and NC5/NC2 were the primer pairs used in these studies (18, 27). Also, studies with TFR3 / TFR4 primers that amplify the 347 bp fragment of the 5.8S gene proved to be particularly useful. The sensitivity of conventional PCR is 1-10 tritrichmonad cells per sample (Felleisen *et al.*, 1998).

Frey *et al.* (17) developed a PCR to amplify trichomonad DNA from a variety of genera like *Tetratrichomonas* sp., for a 379 bp product; *Pentatrichomonas* sp., yielding a 333 bp product; *T. vaginalis* with 363 bp length; and *T. gallinae* at 364 bp. This investigation seems to be very useful in the aspect of reducing false positive results from bovine samples and allows for detecting the "real" causative agent of trichomonad infection.

At this point in time, many studies are conducted with the real-time PCR method because of its better diagnostic accuracy than conventional PCR. A minor groove binder (MGB) probe-based real-time PCR developed by McMillen and Lew (33) amplified the

ITS-1 part within the same rDNA region with a high sensitivity of one trichomonad cell isolated directly from the specimens of cattle and from cell culture. The molecular test was able to detect a single cell per assay from smegma or mucus, which was 2,500 or 250 times more sensitive than microscopy following cultivation from smegma and mucus, respectively, and 500 times more sensitive than culture followed by conventional PCR assay. The efficacy of the MGB probe-based real-time PCR was established on samples from cattle and the summarised results revealed that the method is more sensitive than the cell culture method.

According to Frey *et al*. (16), a diagnostic probe-based real-time PCR which targeted the 5.8S rDNA and flanking ITS-1 and ITS-2 regions of *T. foetus* is able to identify one cell of parasite per sample. However, the molecular test has its limitations and false positive results may occur. Quantitative test results from examination of samples from symptomatic cows revealed the presence of *Tritrichomonas foetus*, but melting peak analysis indicated *Simplicimonas*-like organisms in all "positive" samples. Moreover, no correlation between a positive result and the presence of vaginitis was found. A novel version of this test with truncated primers is more sensitive, with a detection limit of < 1 trophozoite also isolated from a feline faecal sample. The novel real-time PCR also gave no unspecific reaction with *Simplicimonas* sp. (4).

Loop isothermal mediated amplification (LAMP) is the newest method performed for *T. foetus* DNA identification. This amplification test offers rapid diagnosis of infectious diseases without need of sophisticated equipment (36). A LAMP assay targeting the 5.8S rDNA subunit was developed and validated with higher sensitivity (4×10^3 CFU/mL, approximately 10 cells/reaction) than the PCR with TFR 3 and TFR 4 primers (39). LAMP with the elongation factor 1 alpha 1 sequence was performed by Oyhenard (38) for DNA detection of *T. foetus* directly from cervical vaginal mucus without purification of samples. The described test's sensitivity was 100–1,000 times higher than that of cell culture and the previously developed LAMP test for 5.8S ribosomal sequences. Due to the simplicity and cost-effectiveness of the assay compared to PCR or real-time PCR, the LAMP assay may be a good alternative for smaller veterinary clinics with low-skilled staff. Moreover, loop mediated isothermal amplification is more robust for inhibition of reactions.

In 2019 we established LAMP with the β tubulin gene as a target sequence which allowed reliable detection of *T. foetus* in faecal samples from cats. The sensitivity of the LAMP was one trichomonad cell per 150 mg of faeces (4).

Serology

Infection of *T. foetus* in bovines does not develop prominent immune responses. Therefore, only a few serological tests have been established for detection of *T. foetus* antibodies. One is the mucus agglutination test, which is based on the occurrence of specific agglutinin in infected bovine vaginal mucus. However, this method shows relatively low sensitivity and specificity.

An ELISA has been developed for detecting vaginal IgA antibodies against antigen TF1.17. A protective surface antigen (TF1.17) was purified and used in an ELISA for detection of antibodies in vaginal mucus. The advantage of the test is higher specificity, because IgA antibodies remain in high concentration even 24 weeks after infection (25).

Detection of *T. foetus* is also possible by immunohistochemistry with monoclonal antibodies which identify trichomonads from aborted foetus tissue fixed with formalin (45).

T. foetus in parasite hosts – the same or different?

There is much speculation whether *T. foetus* strains isolated from cattle, cats, and pigs are all the same species. Many scientists claim that feline *T. foetus* and bovine/swine *T. foetus* belongs to different species, whereas cattle and pigs may host the same one. Nevertheless, the causative agents of cattle and pig tritrichomonosis are mostly regarded as separate genotypes, even if controversies about their taxonomic relationship have continued for 50 years. Dual names of this pig commensal, *Trichomonas suis* or *Tritrichomonas foetus*, now have currency and the taxonomical status of the parasite is still being discussed.

Morphological studies on bovine and porcine trichomonads conducted in the 1950s and 1960s revealed identical shape, organisation of cells, and size and number of flagella. Based on this detailed analysis, the authors of those studies suggested that these two parasites are identical. Bovine *T. foetus* and swine *T. foetus* (*T. suis*) were also compared at the ultrastructural and biochemical levels by measurements and scanning by electron microscopy, microcinematography, the Thiéry cytochemistry technique for carbohydrate detection and isozyme electrophoresis. In these studies 11 different strains of bovine *T. foetus* and swine *T. foetus* were tested. Cell structure was also screened by scanning and transmission electron microscopy and it was demonstrated that trichomonads from both hosts are morphologically the same. Based on these results and the high degree of isozymatic similarity between the parasites it is suggested that these trichomonads may be different strains of the same species (31).

In 1951 Switzer inoculated cell culture of swine *T. foetus* (*T. suis*) into the vaginal tracts of cows causing infection and infertility (55). Moreover, experimental cross-infection of young pigs with *T. foetus* from cows confirmed the conclusions from previous studies. Five weeks after intranasal inoculation of parasites obtained during necropsy of cows, infection of *T. foetus* in the caecum, small intestine, and nasal cavity of swine was observed (13). Similarly, the preputial cavity of bulls was infected with *T. foetus* isolated from pigs and finally

the viability of one isolate of *T. foetus* in dual hosts was confirmed by successful transmission of this infection by coitus (57). Additionally, in another study, cross-inoculation of cows and pigs with heterologous trichomonads discounted strict host specificity of the parasites (35). Experiments with the sialic-acid binding systems of *T. foetus* (*T. suis*) and *T. foetus* have been carried out to better know their properties. The studies showed that both trichomonads (bovine and swine) have the capacity for adhesion to caecal mucus and for agglutination of human blood cells of groups A1, A2, B, and 0 (40). However, comparison of physiological and metabolic properties at pH 6.4 revealed differences between parasites isolated from the porcine nasal cavities and caeca and the bovine *T. foetus* strains (BP-1). Investigation shows that trichomonads isolated from the nasal cavity and caecum of pigs used glucose, galactose, fructose, mannose, lactose, sucrose, raffinose, and trehalose for their metabolic reactions whereas bovine *T. foetus* used all except lactose and raffinose. In addition, caecal trichomonads were not inhibited by fluoride and 8-hydroxyquinoline but trichomonads from bovine BP-1 strain were (46).

Molecular techniques have great potential to facilitate the study of an organism's origin, especially analysis of its genetic diversity. The experiments conducted by Felleisein (11) with the random amplified polymorphic DNA (RAPD) method gave more consistent answers for the commonality of the bovine *T. foetus* and swine *T. foetus* (*T. suis)* identity by virtue of their exploitation of a molecular technique. In this molecular method 12 different DNA isolates of bovine and porcine strains were tested with 12 different randomly selected sets of primers. Identical banding patterns among different fingerprints of all bovine and swine *T. foetus* from different geographical regions were obtained. Analysis of the genetic diversity of parasites based on RAPD was also continued by Tachezy *et al.* (56) with 29 primers and provided data showing a complex product of about 8–12 fragments which was the same for all bovine and swine trichomonads. Additionally, a dendrogram was constructed based on the RADP results and the high bootstrap values of some internal branches of the bovine/swine *T. foetus* subtree suggested that both strains formed a common branch.

Additionally, the same authors provided RFLP as another molecular tool for *T. foetus* species studies. Digestion of all isolates included in the studies (two bovine and three swine strains) by five restriction endonucleases gave characteristic band patterns of restriction fragments based on the presence of ubiquitous variable-length repetitive sequences in genomic DNA. The patterns were also the same for all tested isolates from cats and pigs (56). Moreover, analysis of small ribosomal subunit RNA (16s rRNA) was made by PCR with primers designed to be complementary to the 16s rRNA target gene. This survey showed that two bovine strains and three swine strains of *T. foetus* had 100% genetic identity. Primers were also designed which amplified parts of variable-length DNA repeats and the identity of bovine and swine *T. foetus* was proved. The results of reactions were common visible bands with the same length (110bp, 210bp, 320bp, and 502bp) in all tested bovine and swine strains.

ITS-1, 5.8S, and ITS-2 sequences are widely used in parasitological investigation as molecular markers for exploring the phylogenetic relationships between several species. Studies conducted by Kleina *et al*. (27) on the reconstruction of Trichomonadidae evolutionary processes with precise employment of phylogenetic inference methods revealed 100% identity between *T. foetus* from cattle and pigs. This conclusion strongly supports all previous studies and indicates that *T. foetus* isolated from cattle and pigs is the same.

One of the greatest contemporary enigmas for parasitologists is the origin of feline *T. foetus* and its relationship to bovine and porcine strains. These parasites were reported in cats for the first time in the late 1990s and were defined as *T. foetus* at that time based on very limited molecular studies and without comparison to *T. foetus* from other animal hosts. Since then several approaches have been attempted to resolve the doubts about the identity or distinctiveness of feline *T. foetus*. Experimental infection of cattle and cats was performed by Stockdale *et al.* (52). In this study heifers were divided into two groups: one group was inoculated with feline *T. foetus* organisms previously cultured from the faeces of a naturally infected cat, and the second group of animals was inoculated with bovine *T. foetus*. The investigation lasted for 11 weeks and during this period cattle were observed and tested for the presence of trichomonads. Comparison of symptoms based on mucus examination and biopsies from the reproductive tract indicated similar but not identical clinical signs of tritrichomonosis in all heifers. The cross-infection investigation was also continued with cats, of which six were inoculated with bovine and one with feline parasites (52). The animal infected by feline *T. foetus* was positive for it in the ileum, caecum, and medial and posterior colon. One of the six cats inoculated with *T. foetus* from cattle was positive in cell culture examination after five weeks and the other animals were negative. Additionally, after necropsy, trichomonads were detected in two cats only in the caecum. It should be noted that during the studies none of the positive cats manifested diarrhoea, weight loss, or fever. The combined results from cross-transmission of *T. foetus* between animal hosts revealed that there are significant differences between the infectivity of bovine and feline isolates. To gain better understanding of molecular aspects of the origin through genetic studies, Šlapeta *et al.* (51) conducted a wide molecular survey with several genes: the internal transcribed spacer regions ITS1 and ITS2, 10 different protein-encoding genes of cysteine proteases 1, 2, and 4–9 (CP1, 2, 4–9), and cytosolic malate dehydrogenase 1 (MDH1). The total amount of nucleotide dissimilarity within all tested markers between feline and bovine isolates of *T. foetus* was 1.03% (47 nucleotide differences/4,552 sequenced

nucleotides). In this comparison between two trichomonad species the most different were the CP2 and CP6 protein-encoding genes of cysteine proteases with 22 (3.29%) and 6 (1.89%) nucleotide differences, respectively, and these seem to be promising potential molecular markers for distinguishing isolates from each host species. Moreover, a single nt mutation (GGA > TGA) in CP5 occurred in the *T. foetus* cat genotype, coding for a Stop codon (TGA), while in the *T. foetus* cattle genotype, GGA codes for the amino acid glycine. In the case of MDH1 there were two nucleotide differences. Direct bidirectional sequencing of the ITS1 and ITS2 regions of four tested feline DNA isolates compared with that of one bovine DNA isolate of *T. foetus* showed a single nucleotide polymorphism (T > C) in the ITS2 region. In the case of bovine and swine trichomonads, the results of the studies confirmed previous hypotheses of the 100% identity of *T. foetus* from cattle and pigs, with a perfect match at nine loci (CP1, 2, 4–9, ITS2, MDH1) except for one isolate of the four tested where a single substitution was identified. Additionally, Šlapeta *et al.* (50) conducted studies with TR7/TR8 primers for amplification of part of the highly variable DNA repeat element. After analysis of PCR products 320 bp in size, 4% identity was indicated (11 conserved nucleotide differences) between feline and bovine *T. foetus* in this particular DNA region.

The genetic diversity of trichomonads from cattle and cats was investigated by Sun *et al.* (54) using the CP8 coding sequence, which is the main gene responsible for parasite-host interaction. Direct sequencing and comparison of CP8 sequences between four feline and two bovine *T. foetus* references revealed two polymorphisms at 168 nt (T > C) and 529 nt (C > A). According to the authors the single nucleotide polymorphism at the 168 nt position was synonymous after translation and the second single nucleotide polymorphism at the 529 nt position was non-synonymous between the cat and cattle isolates.

Additional differences between bovine/swine and feline trichomonads have also been also noted for the quite new molecular marker, elongation factor-1 alpha (EF-1α) gene and for the internal transcribed spacer region 2 (ITS-2) (44), the latter of which was previously published by Felleisein (11). Those two independent genetic loci were selected to be sufficient for PCR sequencing-based genotyping of *T. foetus* isolates from cattle and cats. Sequence alignments of the ITS-2 locus revealed that the feline isolates differed in one nucleotide from the bovine with transition between T and C in all tested samples. In the case of EF-1, the α sequence of feline trichomonads differed from that of bovine trichomonads with five single nucleotide polymorphisms (SNPs). Based on multiple alignments, primers for PCR were developed as a molecular tool for distinguishing the feline *T. foetus* from the bovine. The primer combination used in this method consisted of bovine forward and reverse EF-1α primers.

The PCR test allowed for detection of bovine *T. foetus* with a product size of 460 bp and gave negative results for feline isolates.

Tritrichomonas foetus from cats and cattle was also compared with the results of two-dimensional gel electrophoresis (2DE) coupled with liquid chromatography–tandem mass spectrometry (53). Analysis of the data revealed that the feline and bovine genotypes have highly similar proteomic profiles with only 24 sites where it was possible to indicate the differences between *T. foetus* strains (14 bovine and 10 feline genotypes). Moreover, studies with 2DE zymography and protease-specific fluorogenic substrates showed differences in cysteine protease (CP) expression profiles between the two genotypes. The aim of the subsequent study presented here was deeper analysis of feline and bovine *T. foetus* with a *de novo* RNA-seq approach. In this survey *de novo* analysis with Illumina RNA-seq reads were assembled into two transcriptomes which contained 42,363 and 36,559 contigs for bovine trichomonads and feline trichomonads, respectively. Moreover, 483 bovine and 445 feline transcripts were found as putative proteases by interrogating the MEROPS database. In trichomonads from cattle, CP8 was more often transcribed, and in trichomonads from cats CP7 was the more frequent result. *T. foetus* transcriptomes were similar in size and had similar percentages of GC base pairs. Coding and non-coding regions of the genome libraries revealed high similarities between *T. foetus* from cats and cattle in the functional category of distribution. Analysis of orthologues yielded a large proportion of highly expressed transcripts in both genotypes (bovine genotype: 76%; feline genotype: 56%) with over 50% of the sequences being identified as shared orthologues of the two organisms. Additionally, no biologically significant differences in the functional annotations between the two compared *T. foetus* transcriptomes were found. Moreover, *in silico* analysis proved that when host sequences are considered, drug targets are *T. foetus* species-specific.

Conclusions from this study were that trichomonads from cats and cattle are highly similar and there is strong adaptation to animal hosts. Recent studies conducted by many authors revealed only a minor dissimilarity between the feline, bovine, and swine *T. foetus* suggesting that they may belong to the same species. In order to generate additional information about the phylogeny of *T. foetus*, its genetic diversity in relation to the different host origins of the parasite was investigated at whole genome level (6). In this study the WGS approach was chosen with an available draft genome sequence from bovine *T. foetus* (2) serving as a scaffold for whole genome (re-) sequencing of our bovine, feline, and porcine strains of *T. foetus.* The results obtained in this study showed that only a low degree of indel polymorphism (68 SNPs and indels) was found between the bovine and the swine strains. In the case of the feline and bovine strains there were 65,569 indels from cats and 65,615 indels from pigs detected.

This data was confirmed by PCRs designed to amplify *in silico* selected indel markers, which also showed a distant relationship between bovine, feline, and swine *T. foetus* (6).

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References

1. Bell E.T., Gowan R.A., Lingard A.E., McCoy R.J., Slapeta J., Malik R.: Naturally occurring *Tritrichomonas foetus* infections in Australian cats: 38 cases. J Feline Med Surg 2010, 12, 889–898.
2. Benchimol M., de Almeida L.G., Vasconcelos A.T., de Andrade Rosa I., Bogo M.R., Kist L.W., de Souza W.: Draft genome sequence of *Tritrichomonas foetus* strain K. Genome Announc 2017, 5, e00195-17.
3. Bryan L., Campbell J., Gajadhar A.: Effects of temperature on the survival of *Tritrichomonas foetus* in transport, Diamond's and InPouch TF media. Vet Rec 1999, 144, 227–232.
4. Dąbrowska J., Karamon J., Kochanowski M., Gottstein B., Cencek T., Frey C.F., Muller N.: Development and comparative evaluation of different LAMP and PCR assays for coprological diagnosis of feline tritrichomonosis. Vet Parasitol 2019, 273, 17–23.
5. Dąbrowska J., Karamon J., Kochanowski M., Jędryczko R., Cencek T.: *Tritrichomonas foetus* infection in cat - first detection in Poland. Acta Parasitol 2015, 60, 605–608.
6. Dąbrowska J., Keller I., Karamon J., Kochanowski M., Gottstein B., Cencek T., Frey C.F., Muller N.: Whole genome sequencing of feline strain of *Tritrichomonas foetus* reveals massive genetic differences to bovine and porcine isolates. Int J Parasitol (in press).
7. Doi J., Abe N., Oku Y.: Molecular survey of *Tritrichomonas suis* (= *T. foetus*) 'cat' and 'cattle' genotypes in pigs in Japan. *J Vet Med Sci.* 2012, 12-0377. J Vet Med Sci 2013, 75, 475–479.
8. Doi J., Hirota J., Morita A., Fukushima K., Kamijyo H., Ohta H., Yamasaki M., Takahashi T., Katakura K., Oku Y.: Intestinal *Tritrichomonas suis* (= *T. foetus*) infection in Japanese cats. J Vet Med Sci 2011, 74, 413–417.
9. Dufernez F., Walker R.L., NoEL C., Caby S., Mantini C., Delgado-Viscogliosi P., Ohkuma M., Kudo T., Capron M., Pierce R.J.: Morphological and molecular identification of non-*Tritrichomonas foetus* trichomonad protozoa from the bovine preputial cavity. J Eukaryot Microbiol 2007, 54, 161–168.
10. Erasmus J., De Wet J., Der Merwe H.E., Pienaar G.: Bovine trichomoniasis in the north western Cape Province, western Transvaal and the Orange Free State. Aust Vet J 1989, 53, 470–472.
11. Felleisen R.S.: Comparative genetic analysis of tritrichomonadid protozoa by the random amplified polymorphic DNA technique. Parasitol Res 1997, 84, 153–156.
12. Filho R.B.O., Malta K.C., Borges J.M., Oliveira P.R.F., Filho G., Nascimento G.G., Mota R.A., Junior J.W.P.: Prevalence and risk factors associated with *Tritrichomonas foetus* infection in cattle in the state of Paraiba, Brazil. Acta Parasitol 2018, 63, 346–353.
13. Fitzgerald P.R.: Bovine trichomoniasis. Vet Clin North *Am* Food Anim Pract 1986, 2, 277–282.
14. Foster R.A.: Female reproductive system and mammae. In: *Pathologic basis of veterinary disease*, edited by M.D. McGavin, J.F. Zachary, Elsevier, St. Louis, 2017, pp. 1147–1193.
15. Fox E.W., Hobbs D., Stinson J., Rogers G.M.: A preliminary survey of North Carolina slaughterhouse bulls for *Tritrichomonas foetus*. Bovine Pr 1995, 29, 153–155.
16. Frey C.F., Muller N., Stauber N., Marreros N., Hofmann L., Hentrich B., Hirsbrunner G.: Simplicimonas-like DNA in vaginal swabs of cows and heifers cross-reacting in the real-time PCR for *T. foetus*. Vet Parasitol 2017, 237, 30–36.
17. Frey C.F., Schild M., Hemphill A., Stunzi P., Muller N., Gottstein B., Burgener I.A.: Intestinal *Tritrichomonas foetus* infection in cats in Switzerland detected by *in vitro* cultivation and PCR. Parasitol Res 2009, 104, 783–788.
18. Gookin J.L., Birkenheuer A.J., Breitschwerdt E.B., Levy M.G.: Single-tube nested PCR for detection of *Tritrichomonas foetus* in feline feces. J Clin Microbiol 2002, 40, 4126-4130.
19. Gookin J.L., Breitschwerdt E.B., Levy M.G., Gager R.B., Benrud J.G.: Diarrhea associated with trichomonosis in cats. J Am Vet Med Assoc 1999, 215, 1450–1454.
20. Gookin J.L., Hanrahan K., Levy M.G.: The conundrum of feline trichomonosis. J Feline Med Surg 2017, 19, 261–274.
21. Gookin J.L., Stebbins M.E., Hunt E., Burlone K., Fulton M., Hochel R., Talaat M., Poore M., Levy M.G.: Prevalence of and risk factors for feline *Tritrichomonas foetus* and *Giardia* infection. J Clin Microbiol 2004, 42, 2707–2710.
22. Guven E., Bastem Z., Avcioglu H., Erdem H.: Molecular determination of *Tritrichomonas* spp. in aborted bovine foetuses in Eastern Anatolian Region of Turkey. Vet Parasitol 2013, 196, 278–282.
23. Hibler C.P., Hammond D.M., Caskey F.H., Johnson A.E., Fitzgerald P.R.: The morphology and incidence of the trichomonads of swine, *Tritrichomonas suis* (Gruby & Delafond), *Tritrichomonas rotunda*, n. sp. and *Trichomonas buttreyi*, n. sp. J Protozool 1960, 7, 159–171.
24. Hosein A., Kruth S.A., Pearl D.L., Richardson D., Maggs J.C., Peach H.A., Peregrine A.S.: Isolation of *Tritrichomonas foetus* from cats sampled at a cat clinic, cat shows and a humane society in southern Ontario. J Feline Med Surg 2013, 15, 706–711.
25. Irons P.C., Henton M.M., Bertschinger H.J.: Collection of preputial material by scraping and aspiration for the diagnosis of *Tritrichomonas foetus* in bulls. J S Afr Vet Assoc 2002, 73, 66–69.
26. Kingsbury D., Marks S., Cave N., Grahn R.A.: Identification of *Tritrichomonas foetus* and *Giardia* spp. infection in pedigree show cats in New Zealand. N Z Vet J 2010, 58, 6–10.
27. Kleina P., Bettim-Bandinelli J., Bonatto S.L., Benchimol M., Bogo M.R.: Molecular phylogeny of Trichomonadidae family inferred from ITS-1, 5.8 S rRNA and ITS-2 sequences. Int J Parasitol 2004, 34, 963–970.
28. Koster L.S., Chow C., Yao C.: Trichomonosis in cats with diarrhoea in Hong Kong, China, between 2009 and 2014. JFMS Open Rep 2015, 1, 1–4.
29. Li W., Li W., Gong P., Zhang C., Yang J., Zhang X., Li J.: The prevalence of intestinal trichomonads in Chinese pigs. Vet Parasitol 2015, 211, 12–15.
30. Mancebo O., Russo A., Carabajal L., Monzon C.: Persistence of *Tritrichomonas foetus* in naturally infected cows and heifers in Argentina. Vet Parasitol 1995, 59, 7–11.
31. Mattos A., Sole-Cava A., DeCarli G., Benchimol M.: Fine structure and isozymic characterization of trichomonadid protozoa. Parasitol Res 1997, 83, 290–295.
32. McCool C., Townsend M., Wolfe S., Simpson M., Olm T., Jayawardhana G., Carney J.: Prevalence of bovine veneral disease in the Victoria River District of the Northern Territory: likely economic effects and practicable control measures. Aust Vet J 1988, 65, 153–156.
33. McMillen L., Lew A.E.: Improved detection of *Tritrichomonas foetus* in bovine diagnostic specimens using a novel probe-based real time PCR assay. Vet Parasitol 2006, 141, 204–215.
34. 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.: High prevalence of *Tritrichomonas foetus* infection in Asturiana de la Montaña beef cattle kept in extensive conditions in Northern Spain. Vet J 2012, 193, 146–151.
35. Morgan B.: A summary of research on *Trichomonas foetus*. J Parasitol 1947, 33, 201–206.
36. Mori Y., Notomi T.: Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother 2009, 15, 62–69.
37. Mueller K., Morin-Adeline V., Gilchrist K., Brown G., Šlapeta J.: 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. Vet Parasitol 2015, 212, 105–110.
38. Oyhenart J.: Direct detection of *Tritrichomonas foetus* in cattle genital fluid trough loop mediated isothermal amplification of elongation factor 1 alpha 1. Vet Parasitol 2018, 261, 67–72.
39. Oyhenart J., Martinez F., Ramirez R., Fort M., Breccia J.D.: Loop mediated isothermal amplification of 5.8S rDNA for specific detection of *Tritrichomonas foetus*. Vet Parasitol 2013, 193, 59–65.
40. Pakandl M.: The prevalence of intestinal protozoa in wild and domestic pigs. Vet Med 1994, 39, 377–380.
41. Pareek P., Sharma S., Bishnoi B., Gupta A.: Trichomoniasis in cattle in North West Rajasthan. Indian J Anim Reprod 1994, 15, 27–28.
42. Perez E., Conrad P.A., Hird D., Ortuno A., Chacon J., BonDurant R., Noordhuizen J.: Prevalence and risk factors for *Trichomonas foetus* infection in cattle in northeastern Costa Rica. Prev Vet Med 1992, 14, 155–165.
43. Rae D.O., Crews J.E., Greiner E.C., Donovan G.A.: Epidemiology of *Tritrichomonas foetus* in beef bull populations in Florida. Theriogenology 2004, 61, 605–618.
44. Reinmann K., Muller N., Kuhnert P., Campero C.M., Leitsch D., Hess M., Henning K., Fort M., Muller J., Gottstein B., Frey C.F.: *Tritrichomonas foetus* isolates from cats and cattle show minor genetic differences in unrelated loci ITS-2 and EF-1alpha. Vet Parasitol 2012, 185, 138–144.
45. Rhyan J.C., Wilson K.L., Burgess D.E., Stackhouse L.L., Quinn W.J.: Immunohistochemical detection of *Tritrichomonas foetus* in formalin-fixed, paraffin-embedded sections of bovine placenta and fetal lung. J Vet Diagn Invest 1995, 7, 98–101.
46. Robertson M.: The antigens of *Trichomonas foetus* isolated from cows and pigs. J Hyg 1960, 58, 207–213.
47. Rosypal A.C., Ripley A., Walden H.D.S., Blagburn B.L., Grant D.C., Lindsay D.S.: Survival of a feline isolate of *Tritrichomonas foetus* in water, cat urine, cat food, and cat litter. Vet Parasitol 2012, 185, 279–281.
48. Shi Y., Jiang W., Ma Z., Qiu Y.: A case report of pulmonary tritrichomonosis in a pig. BMC Vet Res 2017, 13, 348.
49. Singh B.N., Lucas J.J., Hayes G.R., Kumar I., Beach D.H., Frajblat M., Gilbert R.O., Sommer U., Costello C.E.: *Tritrichomonas foetus* induces apoptotic cell death in bovine vaginal epithelial cells. Infect Immun 2004, 72, 4151–4158.
50. Šlapeta J., Craig S., McDonell D., Emery D.: *Tritrichomonas foetus* from domestic cats and cattle are genetically distinct. Exp Parasitol 2010, 126, 209–213.
51. Šlapeta J., Muller N., Stack C.M., Walker G., Lew-Tabor A., Tachezy J., Frey C.F.: 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 2012, 42, 1143–1149.
52. Stockdale H.D., Givens M.D., Dykstra C.C., Blagburn B.L.: *Tritrichomonas foetus* infections in surveyed pet cats. Vet Parasitol 2009, 160, 13–17.
53. Stroud L.J., Šlapeta J., Padula M.P., Druery D., Tsiotsioras G., Coorssen J.R., Stack C.M.: Comparative proteomic analysis of two pathogenic *Tritrichomonas foetus* genotypes: there is more to the proteome than meets the eye. Int J Parasitol 2017, 47, 203–213.
54. Sun Z., Stack C., Šlapeta J.: Sequence differences in the diagnostic region of the cysteine protease 8 gene of *Tritrichomonas foetus* parasites of cats and cattle. Vet Parasitol 2012, 186, 445–449.
55. Switzer W.: Atrophic rhinitis and trichomonads. Vet Med 1951, 46, 478-481.
56. Tachezy J., Tachezy R., Hampl V., Šedinova M., Vanacova Š., Vrlík M., Van Ranst M., Flegr J., Kulda J.: Cattle pathogen *Tritrichomonas foetus* (Riedmüller, 1928) and pig commensal *Tritrichomonas suis* (Gruby & Delafond, 1843) belong to the same species. J Eukaryot Microbiol 2002, 49, 154–163.
57. Tachezy J., Tachezy R., Hampl V., Šedinova M., Vanacova Š., Vrlík M., Van Ranst M., Flegr J., Kuldaa J.: Cattle pathogen tritrichomonas foetus (Riedmüller, 1928) and pig commensal *Tritrichomonas suis* (Gruby & Delafond, 1843) belong to the same species. J Eukaryot Microbiol 2002, 49, 154–163.
58. OIE Handistatus, World Animal Health Publication and Handistatus II, Office International des Epizooties, Paris, 2005.
59. Yaeger M., Gookin J.L.: Histologic features associated with *Tritrichomonas foetus*-induced colitis in domestic cats. Vet Pathol 2005, 42, 797–804.
60. Yang N., Cui X., Qian W., Yu S., Liu Q.: Survey of nine abortifacient infectious agents in aborted bovine fetuses from dairy farms in Beijing, China, by PCR. Acta Vet Hung 2012, 60, 83–92.
61. Yao C., Bardsley K., Litzman E., Hall M., Davidson M.: *Tritrichomonas foetus* infection in beef bull populations in Wyoming. J Bacteriol Parasitol 2011, 2, 117. doi:10.4172/2155-9597.1000117
62. Yao C., Koster L.S.: *Tritrichomonas foetus* infection, a cause of chronic diarrhea in the domestic cat. Vet Res 2015, 46, 35.