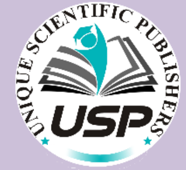
This is an open-access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com


Short Communication https://doi.org/10.47278/journal.ijvs/2025.017

Study of *Tritrichomonas foetus* Representation in Cattle Population in Some Regions of Russia

I.B. Gribchenko [1], N.A. Feofanova [1], N.B. Rakhmatova [2], G.A. Dushanova [2], E.A. Litvinova [1] and V.D. Bets [1,*]

[1]Novosibirsk State Technical University, Faculty of Physics and Technology, 630073, Novosibirsk, Russia
[2]Sharof Rashidov State University, Institute of Biochemistry, Department of Human and Animal Physiology 140100, Samarkand, Uzbekistan
***Corresponding author:** betsvd.bio@gmail.com

Article History: 24-751 Received: 05-Dec-24 Revised: 6-Feb-25 Accepted: 07-Feb-25 Online First: 19-Feb-25

ABSTRACT

Protozoal infection is an important factor in reducing the productivity of farm animals. Pathogenic protozoa are able to affect the gastrointestinal tract and reproductive system. The causative agent of protozoal infection causing abortion and infertility in cattle is most often *Tritrichomonas foetus.* In order to determine the epizootic situation in the region, we studied the representation of pathogenic trichomonads in cattle of the Novosibirsk region. Using specific primers, we detected traces of *T. foetus* DNA in samples obtained from adult animals with clinical signs of endometritis and vaginitis from different farms. Also, vaginal mucus samples from these animals were examined by direct light microscopy, where active trophozoites of *T. foetus* were found, indicating the circulation of the parasitic protozoan in the cattle population of the Novosibirsk region.

Key words: Protozoal infection, *Tritrichomonas foetus*, Cattle.

INTRODUCTION

Obtaining quality and safe products is possible only if the health of farm animals is ensured. And the health of farm animals, in turn, depends on various factors, among which infections play a key role. In addition to bacterial and viral invasion, the gastrointestinal tract, lungs, sexual tracts of mammals and poultry are densely populated with representatives of protozoa. It is important to note that such a set of symbiotic flora was formed over a long time under the action of evolutionary mechanisms. However, in addition to symbionts, there are also pathogenic species of bacteria, fungi and protozoa that can cause infectious process. Protozoal infections cause economic damage to livestock production no less than bacterial or viral infections (Hassan-Kadle et al. 2020).

Since protozoa have adaptation to different antibacterial drugs, the manifestation of their virulence remains not obvious against the background of resistance of some bacterial species and ineffective treatment regimens for animals. Also, protozoa have defense mechanisms against unfavorable conditions, including the formation of cysts, which complicates the process of controlling them (Corliss 2001; Lambrecht et al. 2015; Li et al. 2022). As a consequence, enterprises incur losses from reduced milk yields, live weight, high mortality of young animals and female ulcers, as well as culling of milk and meat (Lianou et al. 2022; Kashif Yar et al. 2023). In addition to economic losses, there is the danger of transmission through farm animal products to humans (Macpherson 2005; Lianou et al. 2022).

Today there are about five causative agents of severe protozoal diseases of humans and animals in the world. Some representatives of parasitic protozoa are found in farm animals, causing mass mortality of livestock and spoilage of products. In cattle, small ruminants, pigs, rabbits and poultry (Mousa et al. 2024), *Cryptosporidium* spp., *Giardia duodenalis, Entamoeba* spp.*, Eimeria* spp. and *Tritrichomonas* spp., *Toxoplasma* spp. are most frequently found in the intestine, and *Tritrichomonas* spp. and *Toxoplasma* spp. are most frequently found in the reproductive system organs (Chudnovskiy et al. 2016). Parasites adhere on the surface of epithelial cells or inside. As a consequence of such interaction, cells become exhausted and die, triggering a cascade of metabolic disorders (Lianou et al. 2022).

Cite This Article as: Gribchenko IB, Feofanova NA, Rakhmatova NB, Dushanova GA, Litvinova EA and Bets VD, 2025. Study of *Tritrichomonas foetus* representation in cattle population in some regions of Russia. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2025.017

Two pathogens, *Cryptosporidium* spp. and *Tritrichomonas foetus*, pose a threat to livestock production worldwide. Cryptosporidiosis most commonly affects the gastrointestinal tract of young animals, resulting in the death of calves, lambs and piglets from dehydration and intoxication, causing serious economic losses to enterprises (De Graaf et al. 1999; Cho and Yoon 2014; Aboelsoued and Abdel Megeed 2022; Mol et al. 2022; Bauer et al. 2023). The parasite is also transmitted from animals to humans, through dairy and meat products contaminated with cysts (Thomson et al. 2017).

But the most unprofitable, from the point of view of enterprise economics, is trichomoniasis caused by the flagellated protozoan *T. foetus*, because animals affected by trichomonads can sharply reduce milk yields, females can hardly cover, and most of the covered animals abort at 3-4 months. In addition, trichomonads are found in the amniotic fluid and rennet of aborted foetuses. Such peculiarities of the parasite tropism may contribute to the development of intrauterine infection of young animals (Florin-Christensen and Schnittger 2018).

Infection occurs sexually, due to artificial insemination with semen from infected bulls, there is also a mechanism of cysts drifting through the gastrointestinal tract, with subsequent introduction into the vaginal mucosa when cysts and trophozoites with feces get on the vulva of cows: during the act of defecation, or when not observing the rules of asepsis during veterinary manipulations (Martínez et al. 2023). After infection, the stage of excystment and the active phase of trophozoite division begins (Mercer and Johnson 2018).

Active trichomonads interact with epithelial cells by adhesion followed by energetic parasitism (Mercer and Johnson 2018). Mechanisms of adhesion have long been considered. For example, for *T. vaginalis,* it has been shown that exosomal vesicles of the parasite induce changes in the host cell and mediate *T. vaginalis-host* interaction by increasing trophozoite adhesion to host cells (Twu et al. 2013; Benchimol et al. 2022; Kochanowsky et al. 2024). Parasite extracellular vesicles contain transport proteins and nucleic acids that are involved in immunomodulation and virulence within the host (Rada et al. 2022). The adhesion mechanism for most of the known *Tritrichomonas* spp. is believed to be the same. In addition, in most protozoa, cell signaling within the population is mediated by exosomes (Mantel and Marti 2014; Marti and Johnson 2016; Szempruch et al. 2016).

The interaction of protozoa with the host within the immune system, is a unique adaptive mechanism for colonization and subsequent active reproduction of the parasite (Barrias et al. 2022). Trichomonads have glycoprotein complexes for communication with host cells, so, for example, *T. vaginalis* after attachment and fusion on the plasma membrane of the epithelial cell, transports lipids and luminal cargo proteins into the host cell (Twu et al. 2013). This communication of exosomal vesicles with ectocervical cells triggers the secretion of pro-inflammatory cytokines interleukin-6 (IL-6) and IL-8 (Chudnovskiy et al. 2016; Szempruch et al. 2016). Such mechanisms may contribute to enhance parasite growth and pathology without eliciting a strong early immune response (Twu et al. 2013).

All this contributes to active colonization of the cervical epithelium and the development of chronic inflammation. For a long time, sick animals do not show clinical symptoms and are asymptomatic carriers, which is associated with difficulties in the diagnosis and treatment of infection (Florin-Christensen and Schnittger 2018).

In addition, trichomonads are also known to actively participate in communication with bacteria and influence the microbiocenosis (Mercer and Johnson 2018). Thus, we have previously shown that in mice infected with intestinal trichomonads, after antibiotic therapy, the intestinal contamination with *Tritrichomonas* spp. increased, while the self-renewal *of Lactobacillus johnsonii* and *Enterococcus faecalis* led to the elimination of trichomonads in a model of induced intestinal dysbiosis (Phukan et al. 2013; Makusheva et al. 2024). Another study showed an inhibitory effect of *Lactobacillus gasseri* on the adhesion of *T. vaginalis* to host cells (Benchimol et al. 2022).

The PCR method is currently used to diagnose trichomoniasis. At the same time, most laboratories still practice the classical method of testing by stained smear, which is ineffective because the method is too inaccurate and depends on the experience of the researcher and subjective factors of smear interpretation. The complexity of therapy also contributes to the spread of protozoal infections, since the only protocol is the use of metronidazole drugs (Love et al. 2017) which have serious side effects with prolonged use, including hepatotoxic effects. Therefore, it remains important to continue the search for effective safe therapeutic agents, as well as active monitoring of protozoa in local and imported farm animal populations throughout the country.

MATERIALS AND METHODS

Ethical statement

The study was conducted in accordance with the principles outlined in the International Recommendations for Biomedical Research Using Animals, developed and published in 1985 by the Council for International Organizations of Medical Sciences and ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Animals

In the study, 52 cows of black-spring Holstein breed from different farms were used, aged from 2 to 4 years, on the 3-7th day after calving, with clinical signs of endometritis: local hyperthermia of the vulva, mucous and mucopurulent discharge from the vagina.

Measurement manipulations

Manipulations were performed before sampling, while the animals were in calm; body temperature was measured using an electronic thermometer (VitaVet PRO, China) pre-treated with petroleum jelly. Respiratory rate was counted per minute, using a phonendoscope applied at the chest. Pulse rate was determined at the median caudal artery. The pulse was examined by palpation on the tail artery with the animal at rest.

Sampling

Samples were collected from animals with signs of endometritis by washing from the vaginal mucosa, using a

In Press

swab (Aptaca S.P.A., Italy), then the swab was placed in a sterile 1.5mL eppendorf (Sovtech, Russia) and transported with refrigerants (4°C) to the laboratory for subsequent DNA extraction. A total of 52 samples were examined.

DNA isolation from swabs

The DU-250 system (Biolabmix, Russia) was used to isolate DNA from swabs. A 700µL of lysis buffer was added to the swab tube and incubated at 70°C for 60min. Next, the samples were centrifuged at 13000rpm for 5min. Then 500µL of supernatant was carefully transferred onto a silica column (comes in the kit). Centrifuged for 30s, 10000rcf. The filtrate was removed. The column was then washed with WB1 buffer by applying 500µL of buffer to the column, followed by centrifugation for 30s, 10000rcf. The filtrate was removed. Repeated washes were performed using WB2 buffer diluted with ethanol (500µL per column, centrifuge 30s, 10000rcf, remove filtrate). Subsequent elution of DNA was performed with elution buffer. The column was transferred to a new 1.5-2mL microtube. A 100µL of EB elution buffer was applied to the center of the column filter and incubated for 3min at room temperature. Then centrifuged for 1min, 10000rcf. An elution volume of 100µL was obtained.

The concentration and purity of isolated DNA were determined using the EzDrop 1000C system (Blue-Ray Biotech, Taiwan). Thus, the concentration of isolated DNA in the samples averaged 69.8ng/µL, and the absorbance (A260/A280) 1.27, respectively.

Real-time PCR

Primers for *Tritrichomonas foetus* were used for PCR (Table 1). All primer sequences were selected using Primer BLAST and Multiple Primer Analyzer program (Ye et al. 2012).

Table 1: Sequence of oligonucleotides used for detection of *Tritrichomonas foetus*

Name of primer	Sequence5'-3'	Source
TRFF1	CGG GTC TTC CTA TAT GAG ACA GAA CC	Felleisen et al. (1998)
TRFR1	CCT GCC GCC GTT GGA TCA GTT TCG TTA A	

The reaction mixture (20µL) contained BioMaster HSqPCR SYBR Blue 1x (BioLabMix, Russia), corresponding primers (300nM each; BIOSSET, Russia) and DNA isolated from vaginal flushes. PCR was performed in a DTlite 4S1 Real Time PCR detection amplifier (DNA Technologies, Russia). DNA was denatured for 5min at 95°C; then 40 cycles were performed: denaturation - 95°C, 15s; primer annealing - 62°C, 25s; elongation - 72°C, 25s.

Normalization of *T. foetus* DNA to total eukaryotic 18S rRNA was performed using the formula: 2^ (Ct of the *Eukaryota* 18S rRNA gene - Ct of the 18S rRNA gene specific for a certain protozoon community), where St is the cycle corresponding to the threshold level of PCR product luminescence.

Gel electrophoresis of DNA samples

Aliquots of PCR amplification products were separated on a 1.5% agarose gel. Briefly, the gel was prepared on 1X TAE buffer (0.5M Tris-acetate, 0.01M EDTA; Medigen, Russia) with addition of ethidium bromide. O'GeneRuler 100bp DNA Ladder Plus marker kit (Fermentas L.S., USA) was used.

To evaluate the efficiency of agarose separation, 10µL of the amplified sample was subjected to electrophoresis for 1h at 100V. The agarose gel images were visualized using a Clinex gel documentation system (Clinex Science Instruments Co., China).

Microscopy

For microscopy, samples with vaginal washings were diluted in Hanks' solution (HBSS, Gibco, USA) cooled to 4°C and a drop was placed on a slide. In addition, the nuclei of parasitic protozoa were stained with Hoechst 33342 fluorescent dye (Thermo FS, USA), for this purpose the samples were diluted in Hanks' solution cooled to 4°C, centrifuged at 1500rpm for 10min, after which the supernatant was removed and 1mL of single phosphate buffer (PBS, Invitrogen, USA) was added, washing in phosphate buffer was repeated two times.

After washing, 10µL of dye at a concentration of 0.1mg/mL was added to 1mL of sample and incubated for 10min, without light. Next, the sample was washed twice in phosphate buffer. After, fixed in 10% formaldehyde, 30min at RT, followed by washing. The samples were examined using an Olympus CX43 direct light microscope with a fluorescence unit (Olympus Corp., Japan).

Statistical treatment of data

Data are presented as mean, error of mean. The normality of distribution was analyzed using the Shapiro-Wilk criterion, and the comparison of two samples with normal distribution was carried out using the parametric Student's t-test. The significance of differences was determined according to $P<0.05$. All calculations were performed in Microsoft Excel 2019 and Past4.

RESULTS

During the research, 52 cows aged from 2 to 4 years with clinical signs of endometritis were examined. Physiological parameters of the animals were studied. According to the results of measurements, pulse rate and respiratory rate in infected animals and animals with negative PCR test results did not show reliable differences (Table 2). All indicators were within the physiological norm. However, body temperature in the two groups was significantly different and was higher in positively reacted animals (Table 2). This may indicate a stronger inflammation in the body. However, the indices of both groups were also within the reference values.

Screening of vaginal wash samples obtained from the examined animals revealed positive cases in 14 out of 52 animals, which is 26.9% (Fig. 1a). In gel electrophoresis in 1.5% agarose gel, the amplification products were 347bp in size (Fig. 1b), which is consistent with literature data (Gharban 2023).

Microscopy of vaginal flushes revealed *T. foetus* trophozoites in small quantities (Fig. 1c). Active forms of trichomonads found in the flushes may indicate contamination of the vaginal mucosa in animals with endometritis.

Table 2: Results of vital signs in cows with positive and negative PCR study

Indicator	Units of measurement	+ (14 in total)	- (38 in total)	P-value
Temperature	°C	39.17±0.26	38.38±0.11	0.0021
Pulse rate	beats/min	67.5±4.86	60.39±2.63	0.1772
Respiratory rate	breath/min	22.35±1.36	21.89±0.75	0.7564

Significance P<0.01.

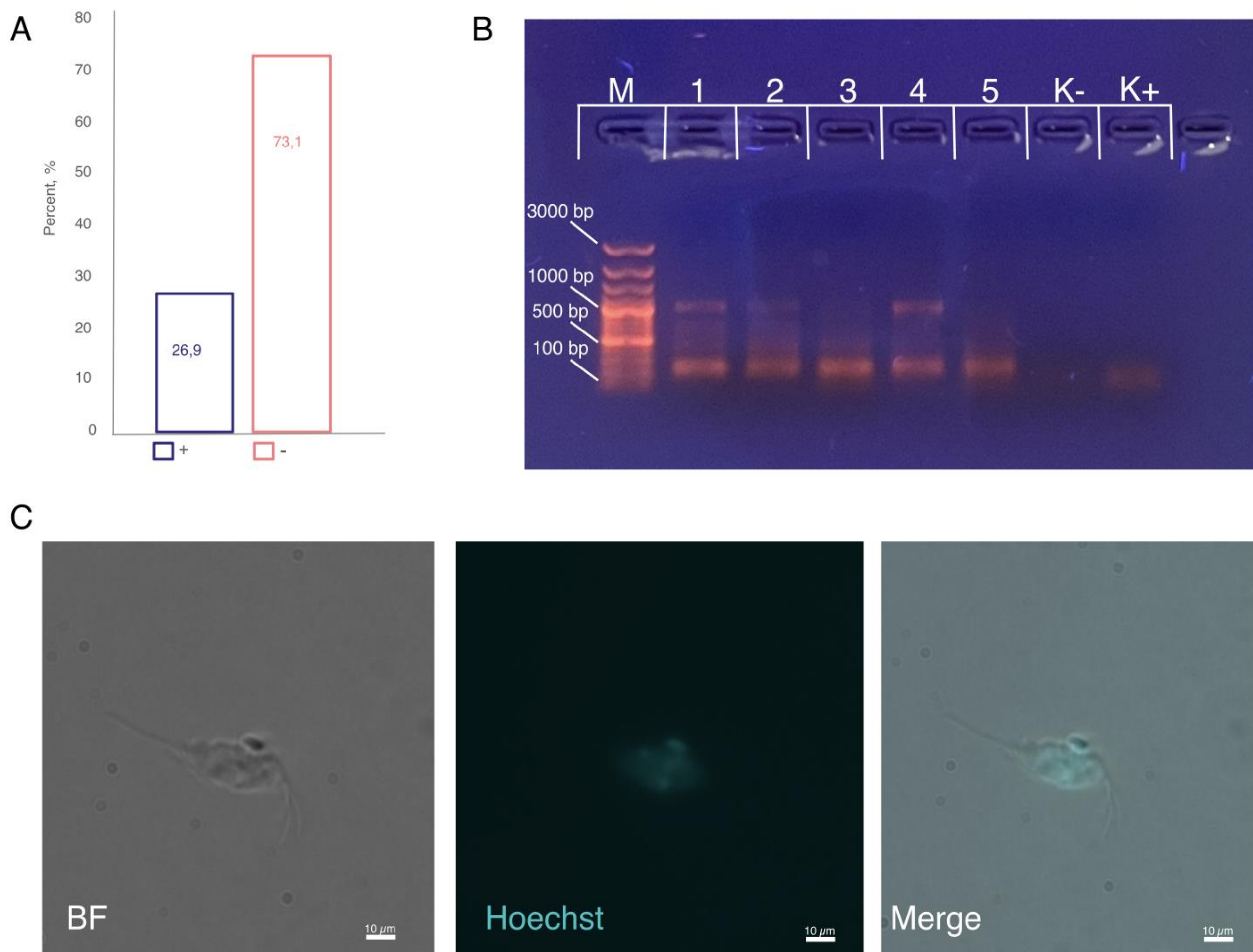


Fig. 1: Detected traces of *T. foetus* protozoan infection in cattle vaginal flush samples. A) Results of testing 52 cows with clinical signs of endometritis, by PCR. B) Representative representation of PCR product length in cow vaginal flush samples by gel electrophoresis in 1.5% agarose gel stained with ethidium bromide (100B and 80A, 1h). Lane (M): Ladder marker (3000-100bp), lane (K+): Positive control, lane (K-): Negative control, lanes (1-5): Positive samples. (C) Trophozoite *of T. foetus* isolated from vaginal mucus. Direct light and fluorescence microscopy, magnification: objective - X40, eyepieces - X10 (Olympus CX43. B).

It is known that treatment regimens with metronidazole were not used in the farms, as opposed to regimens aimed at correcting bacterial communities, which may be associated with protozoan overgrowth. It is important to note, however, that heifers and cows are covered artificially with proven and safe seed.

The question of infection and circulation of trichomonads in the cattle population remains open.

DISCUSSION

According to the data obtained, the protozoosis pathogen *T. foetus* is present in the population of cattle in the Novosibirsk Region. Despite the existing protocols for compulsory diagnostics of animals, protozoa are circulating in the population, which can obviously affect animal performance and their general physiological condition. This may be due to both outdated diagnostic protocols and ineffective preventive measures against protozooses. For timely diagnosis, it is necessary to reduce the intervals between mandatory tests to six months and to perform them in a comprehensive manner, that is, to use molecular methods and microscopy to confirm infection of the vaginal mucosa (or prepuce in men) with active trophozoites. Studies to monitor protozoal infection caused by *T. foetus* are conducted by different groups of scientists. Researchers are selecting methods of different sensitivities to detect the parasite in epithelial flushes. For example, studies have been conducted to assess the prevalence and identify risk factors for infection with the parasite in Poland. The study demonstrated the sensitivity of PCR and loop-mediated isothermal amplification (LAMP) identification, but no spread of infection in cattle was found, only confirmation of parasite circulation in cats and pigs (Dąbrowska et al. 2020).

In a study, Schroeder et al. (2023) described the

development of an updated set of PCR primers and probes that provide increased sensitivity for detection of *T. foetus* in preputial flushes collected in PBS, using real-time reverse transcription real-time PCR (RT-rtPCR). In another study, Jin et al. (2020) obtained molecular characterization of clinical isolates of *T. foetus* in cattle populations in Wyoming, South Dakota and Montana in the US states of Wyoming, South Dakota and Montana. The study showed more than 99% identity of the newly described isolates with other cattle isolates (Jin et al. 2020).

In Iraq, a team of scientists also conducted a monitoring study on the presence of *T. foetus* in the cattle population, where it was shown that almost 21% of aborted cows had a positive PCR test. And scientists also showed that *T. foetus* isolates showed high identity with Thai (MN560972.2) and Chinese (MH115435.1) isolates (Gharban 2023). In Australia, contamination of cattle populations in an extensive livestock production area with logistical constraints has been shown. Thus, the parasite was detected in preputial flushes of bovines by PCR followed by genotyping (Calvani et al. 2021).

In large cities and rural areas, great attention needs to be paid to contamination of domestic animals by the *T. foetus* parasite, including cats. Several studies have shown screening of domestic cats for the presence of trichomonads. As a result, it has been shown that most animals with clinical signs of gastrointestinal disease were contaminated with *T. foetus* (Leelanupat et al. 2020; Crisi et al. 2021). Disease monitoring using modern molecular methods allows maintaining an adequate epizootic picture of the region. More and more studies in this direction are appearing and molecular diagnostic methods are being improved as the most sensitive ones. At the same time, it is important to publish such data to understand the prevalence of protozoosis and its modifications.

Conclusion

The spread of protozoal infections is known to be associated with ineffective therapeutic measures. At the same time, protozoal infections remain underestimated in terms of their scale and the nature of economic damage caused. Continuous monitoring of disease incidence and search for new effective and safe drugs will reduce economic losses in livestock production.

Conflict of interest: The authors declare that there is no conflict of interest.

Acknowledgements: The study was supported by a grant from the Russian Science Foundation (project No. 23-26-00270).

Author's Contribution: Conceptualization - EA Litvinova, VD Bets; Data curation - VD Bets, IB Gribchenko; Formal analysis - IB Gribchenko, NA Feofanova, NB Rakhmatova; Funding acquisition - NA Feofanova; Investigation - IB Gribchenko, NB Rakhmatova, GA Dushanova; Methodology - GA Dushanova, EA Litvinova; Project administration - VD Bets; Supervision - EA Litvinova; Validation - EA Litvinova, GA Dushanova; Visualization - VD Bets, NA Feofanova; Writing – original draft - IB Gribchenko; Writing – review and editing - VD Bets.

REFERENCES

Aboelsoued D and Abdel Megeed KN, 2022. Diagnosis and control of cryptosporidiosis in farm animals. Journal of Parasitic Diseases 46: 1133–1146. https://doi.org/10.1007/s12639-022-01513-2

Barrias E, Zuma A and Souza W, 2022. *Trichomonas vaginalis*: Lifestyle, cellular biology, and molecular mechanisms of pathogenesis. Lifecycles of Pathogenic Protists in Humans 35: 541-617. https://doi.org/10.1007/978-3-030-80682-8

Bauer J, Kaske M, Oehm A and Schnyder M, 2023. Dynamics of protozoal excretion in the faeces of calves during the first 28 days after arrival at the fattening farm indicate infection before regrouping and show poor temporal correlation with diarrhoea. Parasites and Vectors 16: 338. https://doi.org/10.1186/s13071-023-05911-0

Benchimol M, Gadelha AP and de Souza W, 2022. Unusual Cell Structures and Organelles in *Giardia intestinalis* and *Trichomonas vaginalis* Are Potential Drug Targets. Microorganisms 10: 2176. https://doi.org/10.3390/MICROORGANISMS10112176

Calvani NED, Šlapeta J, Onizawa E, Eamens K, Jenkins C and Westman ME, 2021. Not gone but forgotten: *Tritrichomonas foetus* in extensively-managed bulls from Australia's Northern Territory. Current Research in Parasitology and Vector Borne Diseases 2021: 100012. https://doi.org/10.1016/j.crpvbd.2021.100012

Cho YI and Yoon KJ, 2014. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. Journal of Veterinary Science 15(1): 1–17. https://doi.org/10.4142/jvs.2014.15.1.1

Chudnovskiy A, Mortha A, Kana V, Kennard A, Ramirez JD, Rahman A, Remark R, Mogno I, Ng R, Gnjatic S, Amir E ad D, Solovyov A, Greenbaum B, Clemente J, Faith J, Belkaid Y, Grigg ME and Merad M, 2016. Host-Protozoan Interactions Protect from Mucosal Infections through Activation of the Inflammasome. Cell 167: 444-456. https://doi.org/10.1016/j.cell.2016.08.076

Corliss JO, 2001. Protozoan Cysts and Spores. In Encyclopedia of Life Sciences: 1-8. https://doi.org/10.1038/npg.els.0001934

Crisi PE, Paoletti B, Morelli S, Simonato G, Colombo M, Tiscar PG and Boari A, 2021. *Tritrichomonas foetus* in cats from Central Italy: Clinical signs and risk factors. Veterinary Parasitology and Regional Studies Reports 24: 100577. https://doi.org/10.1016/j.vprsr.2021.100577

Dąbrowska J, Karamon J, Kochanowski M, Sroka J, Skrzypek K, Zdybel J, Różycki M, Jabłoński A and Cencek T, 2020. *Tritrichomonas Foetus*: A study of prevalence in animal hosts in Poland. Pathogens 9(3): 203. https://doi.org/10.3390/pathogens9030203

De Graaf DC, Vanopdenbosch E, Ortega-Mora LM, Abbassi H and Peeters JE, 1999. A review of the importance of cryptosporidiosis in farm animals. International Journal for Parasitology 29: 1269–1287. https://doi.org/10.1016/S0020-7519(99)00076-4

Felleisen RSJ, 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. https://doi.org/10.1128/jcm.36.2.513-519.1998

Florin-Christensen M and Schnittger L, 2018. Parasitic protozoa of farm animals and pets. In Parasitic Protozoa of Farm Animals and Pets. Springer International Publishing, Cham, Switzerland, pp: 313-388. https://doi.org/10.1007/978-3-319-70132-5

In-Press

Gharban HAJ, 2023. Molecular prevalence and phylogenetic confirmation of bovine trichomoniasis in aborted cows in Iraq. Veterinary World 16(3): 580–587. https://doi.org/10.14202/vetworld.2023.580-587

Hassan-Kadle AA, Ibrahim AM, Nyingilili HS, Yusuf AA and Vieira RFC, 2020. Parasitological and molecular detection of *Trypanosoma spp.* in cattle, goats and sheep in Somalia. Parasitology 147(14): 1786–1791. https://doi.org/10.1017/S003118202000178X

Jin Y, Du A and Yao C, 2020. Clinical isolates of *Tritrichomonas foetus* in bulls in Wyoming, South Dakota and Montana, USA. Veterinary Research 16: 12. https://doi.org/10.1186/s12917-020-2229-6

Kashif Yar M, Mahmood M, Ijaz M, Hayat Jaspal M, Rafique Z, Hussain Badar I and Rafique K, 2023. Effect of cattle-specific diseases on carcass inspection and meat quality. Cattle Diseases - Molecular and biochemical approach. Veterinary Medicine and Science. IntechOpen, London, UK. 16: 33-48. https://doi.org/10.5772/intechopen.110384

Kochanowsky JA, Mira PM, Elikaee S, Muratore K, Rai AK, Riestra AM and Johnson PJ, 2024. *Trichomonas vaginalis* extracellular vesicles up-regulate and directly transfer adherence factors promoting host cell colonization. Proceedings of the National Academy of Sciences of the United States of America 121(25): e2401159121. https://doi.org/10.1073/pnas.2401159121

Lambrecht E, Baré J, Chavatte N, Bert W, Sabbe K and Houf K, 2015. Protozoan cysts act as a survival niche and protective shelter for foodborne pathogenic bacteria. Applied and Environmental Microbiology 81: 5604–5612. https://doi.org/10.1128/AEM.01031-15

Leelanupat A, Kamyingkird K, Chimnoi W and Nimsuphan B, 2020. Prevalence of *Tritrichomonas foetus* infection in cats in Bangkok metropolitan area and in vitro drug sensitivity testing. Veterinary Parasitology and Regional Studies Reports 21: 100440. https://doi.org/10.1016/j.vprsr.2020.100440

Lianou DT, Arsenopoulos KV, Michael CK, Papadopoulos E and Fthenakis GC, 2022. Protozoan Parasites in Adult Dairy Small Ruminants and Potential Predictors for Their Presence in Faecal Samples. Microorganisms 10: 1931. https://doi.org/10.3390/microorganisms10101931

Li Y, Wang Y, Zhang S, Maurer-Alcalá XX and Yan Y, 2022. How Ciliated Protists Survive by Cysts: Some Key Points During Encystment and Excystment. In Frontiers in Microbiology 13: 785502. https://doi.org/10.3389/fmicb.2022.785502

Love D, Fajt VR, Hairgrove T, Jones M and Thompson JA, 2017. Metronidazole for the treatment of *Tritrichomonas foetus* in bulls. BMC Veterinary Research 13: 107. https://doi.org/10.1186/s12917-017-0999-2

Macpherson CNL, 2005. Human behaviour and the epidemiology of parasitic zoonoses. In International Journal for Parasitology 35: 1319–1331. https://doi.org/10.1016/j.ijpara.2005.06.004

Makusheva Y, Goncharova E, Bets V, Korel A, Arzhanova E and Litvinova E, 2024. Restoration of *Lactobacillus johnsonii* and *Enterococcus faecalis* caused the elimination of *Tritrichomonas sp.* in a model of antibiotic-induced dysbiosis. International Journal of Molecular Sciences 25 (10): 5090. https://doi.org/10.3390/ijms25105090

Mantel PY and Marti M, 2014. The role of extracellular vesicles in Plasmodium and other protozoan parasites. Cellular Microbiology 16: 344–354. https://doi.org/10.1111/cmi.12259

Marti M and Johnson PJ, 2016. Emerging roles for extracellular vesicles in parasitic infections. Current Opinion in Microbiology 32: 66–70. https://doi.org/10.1016/J.MIB.2016.04.008

Martínez CI, Iriarte LS, Salas N, Alonso AM, Pruzzo CI, dos Santos Melo T, Pereira-Neves A, de Miguel N and Coceres VM, 2023. Prolonged survival of venereal *Tritrichomonas foetus* parasite in the gastrointestinal tract, bovine fecal extract, and water. Microbiology Spectrum 11: e00429-23. https://doi.org/10.1128/SPECTRUM.00429-23

Mercer F and Johnson PJ, 2018. *Trichomonas vaginalis*: Pathogenesis, Symbiont interactions, and host cell immune responses. Trends in Parasitology 34: 683–693. https://doi.org/10.1016/j.pt.2018.05.006

Mol W, Clinquart J, Pas ML, Bokma J and Pardon B, 2022. Pathogen-oriented approaches for neonatal calf diarrhea. In Vlaams Diergeneeskundig Tijdschrift 91(4): 167–181. https://doi.org/10.21825/vdt.85202

Mousa MR, Attia MM, Salem HM, Al-Hoshani N, Thabit H, Ibrahim MA, Albohiri HH, Khan SA, El-Saadony MT, El-Tarabily KA and El-Saied MA, 2024. Coinfection of the gut with protozoal and metazoal parasites in broiler and laying chickens. Poultry Science 103: 103227. https://doi.org/10.1016/j.psj.2023.103227

Phukan N, Parsamand T, Brooks AES, Nguyen TNM and Simoes-Barbosa A, 2013. The adherence of *Trichomonas vaginalis* to host ectocervical cells is influenced by lactobacilli. Sexually Transmitted Infections 89: 455–459. https://doi.org/10.1136/sextrans-2013-051039

Rada P, Hrdý I, Zdrha A, Narayanasamy RK, Smutná T, Horáčková J, Harant K, Beneš V, Ong SC, Tsai CY, Luo HW, Chiu CH, Tang P and Tachezy J, 2022. Double-Stranded RNA Viruses Are Released From *Trichomonas vaginalis* Inside Small Extracellular Vesicles and Modulate the Exosomal Cargo. Frontiers in Microbiology 13: 893692. https://doi.org/10.3389/fmicb.2022.893692

Schroeder ME, Meza D, Shah R, Leyva-Baca I, Conrad R and Ferro PJ, 2023. Detection of *Tritrichomonas foetus* by RT-rtPCR in pooled bovine preputial washings. Journal of Veterinary Diagnostics and Investigations 35(2): 178-181. https://doi.org/10.1177/10406387221149407

Szempruch AJ, Dennison L, Kieft R, Harrington JM and Hajduk SL, 2016. Sending a message: Extracellular vesicles of pathogenic protozoan parasites. Nature Reviews Microbiology 14: 669–675. https://doi.org/10.1038/nrmicro.2016.110

Thomson S, Hamilton CA, Hope JC, Katzer F, Mabbott NA, Morrison LJ and Innes EA, 2017. Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. Veterinary Research 48: 42. https://doi.org/10.1186/s13567-017-0447-0

Twu O, de Miguel N, Lustig G, Stevens GC, Vashisht AA, Wohlschlegel JA and Johnson PJ, 2013. *Trichomonas vaginalis* exosomes deliver cargo to host cells and mediate host: Parasite interactions. PLoS Pathogens 9: e1003482. https://doi.org/10.1371/journal.ppat.1003482

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S and Madden TL, 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13: 134. https://doi.org/10.1186/1471-2105-13-134