

Bovine trichomoniasis: a review

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Abstract

Trichomoniasis is a bovine venereal disease that causes substantial economic losses. Bulls serve as asymptomatic carriers for the protozoan *Tritrichomonas foetus*, whereas infection in females may result in early embryonic death, abortion, pyometra, fetal maceration, or infertility, all of which negatively influence the profitability of a cattle operation. When allowed adequate recovery time following infection most females mount an immune response and return to normal reproductive status. However, the male can remain infected and remain a risk to a producer's breeding program. Currently no legal treatment for this disease exists in the United States therefore veterinarians and cattle producers must focus on preventive management and surveillance measures such as testing, identification and removal of positive animals. Understanding the pathogenesis, prevalence, economic impact, and diagnosis of trichomoniasis will assist with implementation of appropriate prevention and control programs. This paper reviews the pathogenesis, prevalence, economic impact, and diagnosis of trichomoniasis in cattle, as well as common guidelines for the prevention and control of trichomoniasis.

Keywords: Trichomoniasis, *Tritrichomonas foetus*, epithelial crypts.

Introduction

The bovine venereal disease trichomoniasis is caused by the protozoan *Tritrichomonas foetus* (*T. foetus*). Bulls serve as asymptomatic carriers when the organism colonizes the epithelium of the penis or prepuce with no clinical signs. Following coitus or artificial insemination with contaminated semen females develop uterine infections that may lead to early embryonic death, abortion, fetal maceration, pyometra or infertility. This manuscript reviews the pathophysiology of bovine trichomoniasis as well as common guidelines for diagnosis

Venereal Disease

T. foetus is an obligate parasite of the bovine reproductive tract. Similar to most venereal diseases, the male is an asymptomatic carrier while the female suffers identifiable consequences of infection. *T. foetus* in bulls localizes in the smegma (secretions) of the epithelial lining of the penis, prepuce, and distal urethra.¹ The organism does not invade the epithelium, and therefore does not invoke an immune response in the bull.² *T. foetus* causes no penile or preputial lesions and does not affect libido.^{3,4} There are no observable changes in semen quality attributable to the organism's presence, however in a recent study by Benchimol, et al, exposure to *T. foetus* resulted in decreased spermatozoal motility, agglutination of sperm cells, and eventual phagocytosis.⁵⁻⁷ The only clinical sign that may be observed in an infected bull is a mild transient preputial discharge during the first two weeks of an infection.⁴

Infection in young bulls (less than 3-4 years of age) is purportedly most often transient, with disease transmission only occurring if sexual contact with a non-infected cow occurs within minutes to days following breeding of an infected cow.^{8,9} Studies by

Morgan⁸ and Clark⁹ indicate that clearance of the organism in a young bull is possible within 20 minutes following breeding an infected cow. Transmission of *T. foetus* by a young bull is therefore likely to be a passive, mechanical transmission that differs from transmission associated with a chronically infected older bull.

T. foetus infection in the cow occurs during coitus with an infected bull. The organism transverses the cervix and colonizes the entire reproductive tract within 1-2 weeks,¹⁰ and as the organism multiplies in the uterus it can cause death of the embryo or fetus, most commonly between gestational days 15 to 80.¹¹ Pyometra and abortion are often the first physical signs of trichomoniasis noticed in a herd, but these signs occur in fewer than 5% of infected animals.¹² Infertility due to embryonic death is the most economically damaging clinical sign and occurs in a larger percentage of infected cows. An affected cow's interestrus interval is usually prolonged because the embryonic loss typically occurs after maternal recognition of pregnancy (days 15-17 of gestation).¹⁰ Unlike the bull, the cow typically mounts an effective immune response to *T. foetus*,⁴ but the time it takes to clear *T. foetus* from the cow's reproductive tract is quite variable. Primary infections may be cleared from the reproductive tract in as little as 95 days¹³ or as long as 22 months.⁸ Subsequent infections are cleared in about 20 days, indicating an anamnestic response.⁹ Immunity does not persist, however, and the anamnestic response is only significant if re-infection occurs within about 15 months of the primary infection.^{14,15} A cow in a herd with a long breeding season could therefore become pregnant and infected with *T. foetus* early in the breeding season, lose that embryo, be infertile for several months, clear the initial *T. foetus* infection, rebreed, conceive, and carry a calf to term as a result of temporary immunity. The result is that more cows will

calve later in the calving season than desired, and there is a resultant wide variety in weaning weights rather than just a reduced calving percentage. The later-born calves are then marketed at lighter weights, or the cattle producer will incur increased feeding costs to achieve a desired market weight. In either case the cattle producer will sustain substantial economic losses.

Economic aspects

Economic losses due to venereal disease result from culling and replacement of infected animals, a decreased percentage of cows calving or calving later than desired with subsequent reduced calf crop and lower weaning weight caused by failure or delay of conception.¹⁴ Fitzgerald, et al¹⁶ estimated in 1958 that each infected bull in a large herd was responsible for an \$800 loss per year. Wilson, et al¹⁷ estimated a \$2.5 million annual calf loss in 1979 due to trichomoniasis in Oklahoma replacement heifers. In 1986, Fitzgerald estimated that the total economic impact in the USA was \$65 million annually.¹⁸ In a 1991 study, Speer, et al estimated that annual losses could reach near \$650 million.¹⁹ Recently the state of Louisiana estimates that current losses exceed \$100 million for that state alone, so the economic loss is likely considerably greater than earlier studies.²⁰

During the 1990's the Idaho legislature approved statutes that prescribe regulations for identifying and eliminating *T. foetus* bulls within the state and for importation of bulls into that state.²¹ Since that time other states have adopted similar legislation and currently the states of Nebraska, North Dakota, South Dakota, Montana, Wyoming, Idaho, Washington, Oregon and Utah require bulls be test negative for trichomoniasis before being transported into the state, sold, or used on public land.²²⁻²⁹

The Texas Animal Health Commission has recently passed similar requirements³⁰. These regulations reflect the growing concern for control of this bovine venereal disease which is so economically important in the United States.

Diagnosis of Bovine Trichomoniasis

Diagnosis of *T. foetus* has traditionally relied upon microscopic identification of key morphological characteristics in preputial smegma or cervicovaginal mucus (CVM) incubated in various culture media. Such characteristics include three anterior flagella, one posterior flagellum, and an undulating membrane resulting in a jerky movement pattern. However, accurate microscopic identification of *T. foetus* can be complicated by the presence of other trichomonadid protozoa.³¹⁻³⁵ Contamination of the preputial orifice, prepuce, or penis with fecal material probably explains the presence of these opportunistic trichomonads. Several non-pathogenic protozoa are normal inhabitants of the bovine gastrointestinal tract,³⁶⁻³⁸ and therefore proper cleaning of the preputial orifice and proper sampling techniques are critical to avoid fecal contamination of diagnostic samples. None of the contaminating trichomonads, however, results in reproductive pathology in cows or bulls.³⁹ Therefore, research has recently focused on molecular-based assays to accurately differentiate *T. foetus*^{33,40-42} from other trichomonads. Given the lack of legal therapy for bulls infected with *T. foetus* in the United States the only reasonable course of action is to slaughter an infected bull. It is therefore imperative to correctly identify *T. foetus*-infected bulls and not misdiagnose based on the presence of non-pathogenic fecal trichomonads.

At present, molecular-based assays are most commonly used as confirmatory tests for bovine trichomoniasis because of the relatively low cost of *in vitro* cultivation

compared to molecular-based assays. However, molecular-based assays are currently very effective in diagnosing human trichomoniasis caused by *Trichomonas vaginalis*, with a sensitivity of 95% and a specificity of 98%.⁴³ It is therefore very likely that in the future the preferred diagnostic test for bovine trichomoniasis will be a molecular-based assay, and some researchers have already advocated their use as an independent diagnostic test for bovine trichomoniasis.^{44,45}

Sampling techniques for detection of trichomoniasis in the male

Several sampling techniques are utilized for obtaining diagnostic specimens in the bull including: 1) a swab technique;⁴⁶ 2) a dry pipette technique;^{9,47} 3) a wet pipette technique;⁴⁸ and 4) the douche technique.⁴⁸ Fitzgerald, et al compared the swab and pipette techniques and reported that the number of parasites recovered via the swab technique is only 20% of the number of parasites recovered via pipette scraping.⁴⁹ The swab technique is therefore rarely used in the United States. The dry pipette technique is one of the most common sampling methods in the U SA, while the douche method is the preferred technique in Europe.⁴⁷ Schönmann, et al reported that the two methods are not statistically different.⁴⁷

Regardless of technique used, it is generally recommended that bulls be sexually rested 1-2 weeks before testing for *T. foetus*; otherwise, false-negative results are more likely because breeding mechanically removes many of the organisms from a bull's penis and prepuce. Given the sensitivity of *T. foetus* cultures, false-negative results are also possible even if a bull has been sexually rested. Only with three negative tests at weekly intervals (Figure 1) can a veterinarian or producer be 99% sure that a bull is *T. foetus* negative.⁵⁰

	<u>Result</u>	<u>Sensitivity (in series)</u>
First test	Negative	80%
Second test (one week later)	Negative	96%
Third test (one week later)	Negative	99

Figure 1. Sensitivity (in series) of *T. foetus* cultures.⁵⁰

Sampling techniques for diagnosis of trichomoniasis in the female

Researchers investigating diagnostic sampling methodologies for *T. foetus* have focused primarily on optimizing sample collection and culture from bulls because of their propensity to develop chronic infections. The technique most commonly used to sample female cattle for *T. foetus* is a dry pipette technique.⁴⁸ An infusion pipette is used to aspirate CVM from the vaginal fornix or near the external cervical os. Alternatively, in the case of a post-coital pyometra, an infusion pipette can also be used to aspirate some of the content of the uterus. Either sample is then examined directly or placed into appropriate culture medium. Culturing *T. foetus* from CVM has a reported sensitivity of 58 to 75%.⁵¹ Samples can also be evaluated with appropriate molecular-based assays.

In vitro culture of *Trichomoniasis foetus*

Direct microscopic examination of specimens for *T. foetus* may be diagnostic, but a far more sensitive method for the detection of *T. foetus* is *in vitro* culture of preputial smegma in a selective nutrient medium for up to a week.⁵¹⁻⁵³ *In vitro* culture allows the proliferation of *T. foetus* to more readily detectable levels. All cultures containing

organisms resembling *T. foetus* should be confirmed with appropriate molecular-based assays to avoid false-positive results due to fecal trichomonad contamination of culture media.^{31,32,54} Alternatively, samples may be submitted directly for molecular-based evaluation. If polymerase chain reaction-based evaluations are not available, a current study by Corbeil, et al suggest that immunofluorescent assay may be useful in the diagnosis of *T. foetus*.⁵⁵

In vitro culture media

Various culture and transport media systems have been used including Kupferberg medium and broth, Claussen's medium, Sutherland medium, trypticase-yeast extract-maltose (TYM) medium, Diamond's medium, and most recently the InPouch® TF (BioMed Diagnostics, White City, OR, USA) *Trichomonas foetus* culture pouch. *In vitro* cultivation using either Diamond's medium or the InPouch® TF is currently the most common method used to diagnose *T. foetus* in the United States. Both culture systems are fairly equal in sensitivity.^{47,56-58} However, the InPouch® TF is somewhat more convenient than Diamond's medium.⁵⁹ The InPouch® TF has a 12-month shelf-life at room temperature, compared to a much shorter refrigerator-life for Diamond's medium. Also, the plastic pouch design of the InPouch® TF is less likely to break or leak than tubes containing Diamond's medium. Unfortunately, the InPouch® TF is more expensive than Diamond's medium.

For many years, cultivation of microorganisms with motility and morphology resembling *T. foetus* in either the InPouch® TF or Diamond's medium was considered to be 100% specific. However, accurate microscopic identification of *T. foetus* has since been shown to be complicated by the presence of other contaminating trichomonadid

protozoa. All cultures containing organisms resembling *T. foetus* should therefore be confirmed with appropriate molecular-based assays, or samples should be submitted directly to a laboratory for molecular analysis. Contact the laboratory prior to sample collection to verify the appropriate transport medium.

Treatment of cattle infected with *Tritrichomoniasis foetus*

One of the complicating factors associated with bovine trichomoniasis is that there are currently no effective treatments with U.S. Food and Drug Administration approval. Historically, the most successful treatment for bulls with trichomoniasis involved systemic treatment with nitromidazole derivatives.^{51,60-62} Despite its effectiveness, the use of nitromidazole derivatives is now illegal in food-producing animals in the U.S. because of their mutagenic and carcinogenic properties, and no alternative treatments are available. However, a recent study by Carvalho, et al⁶³ found that *T. foetus* exposed *in vitro* to mebendazole resulted in internalization of the flagella, disruption of the nucleus, and cytoplasmic vacuolization. These findings suggest new possibilities in the treatment of trichomoniasis. Still, the lack of effective approved therapies for bovine trichomoniasis emphasizes the need for appropriate preventive and control measures.

Prevention and Control of Bovine Trichomoniasis

Preventing the introduction of *T. foetus* into a cattle herd and controlling trichomoniasis in an infected herd follow many of the same management strategies and to a large extent focus on herd biosecurity. Ideally, every cattle operation should focus on preventing the introduction of *T. foetus*.

Recommended practices to prevent the introduction of *T. foetus* into a cattle herd include:

- 1) When possible, avoid grazing cattle on public lands where both bulls and cows have a much greater risk of exposure through coitus with other *T. foetus*-infected animals.⁶⁴
- 2) Utilize artificial insemination when possible
- 3) Cull all open cows and heifers.
- 4) Control animal movement into a herd. Maintain good fences to prevent *T. foetus*-infected animals from inadvertently entering a herd, or to prevent uninfected animals from temporarily entering a *T. foetus*-infected herd and then returning with *T. foetus* to their uninfected herd of origin.
- 5) Purchase virgin bulls and heifers as replacements. Buying older bulls and cows as replacements greatly increases the chance of purchasing a *T. foetus*-infected animal. While older bulls are much more likely to become chronically infected with *T. foetus* than cows, a small percentage of cows will also become chronically infected.
- 6) Test bulls for *T. foetus* at least once before introducing them into a new herd
- 7) The test should be performed after two weeks of sexual rest. Ideally, a bull should have three negative cultures at weekly intervals.
- 8) Maintain as young a bull battery as possible. Older bulls are considered more likely to develop chronic *T. foetus* infections. However, any bull exposed to *T. foetus* in a natural breeding situation is capable of becoming chronically infected, regardless of age.

- 9) Breed purchased cows and heifers in a separate herd, and cull all open animals.

Ideally, continue to keep the pregnant animals segregated from the rest of the herd through the next breeding season.

- 10) Consider immunization against *T. foetus* in high-risk herds.

Recommendations for control of trichomoniasis in an infected herd includes:

- 1) Test and cull all infected bulls. Infected bulls should be sold for slaughter only.
- 2) Decrease the number of bulls per breeding unit. Single-sire herds offer the lowest exposure potential. However, single-sire units may not always be practical.
- 3) Reduce the average age of the bull herd. Older bulls are considered more likely to develop chronic *T. foetus* infections. However, any bull exposed to *T. foetus* in a natural breeding situation is capable of becoming chronically infected, regardless of age.
- 4) Test bulls for *T. foetus* at least once before introducing them into a new herd. The test should be performed after two weeks of sexual rest. Ideally, a bull should have three negative cultures at weekly intervals.
- 5) Utilize artificial insemination when possible.
- 6) Reduce the breeding season to 60-90 days and cull all open cows and heifers. If there are too many open cows for culling to be economically feasible, then at least these animals should be separated into a high-risk herd. A long breeding season not only allows propagation of *T. foetus*, but it may also hide production losses due to reduced weaning weights because of delayed conception.
- 7) Culture all cases of pyometra diagnosed in cows or heifers during pregnancy examinations.

8) Submit all aborted fetuses and placental tissue to a diagnostic laboratory.

Immunization against *T. foetus* is an extremely important management tool for herds infected with *T. foetus*. Research trials clearly demonstrate the benefit of *T. foetus* vaccination.⁶⁴⁻⁶⁶ TrichGuard® (Fort Dodge Animal Health, Fort Dodge, IA, USA) and TrichGuard® V5L (Fort Dodge Animal Health) are currently the only *T. foetus* vaccines available in the United States. The vaccines require an initial subcutaneous dose followed by a booster dose two to four weeks later. The second injection should precede the breeding season by four weeks. Annual revaccination four weeks prior to the breeding season is recommended.

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