Trichomoniasis in Beef Herds

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Abstract

Reproductive efficiency is one of the most important factors influencing the economic success of a cow-calf operation. Bovine trichomoniasis, also known as trichomonosis, is a venereal disease caused by the protozoan *Tritrichomonas foetus* that negatively impacts a herd's reproductive efficiency, thereby having a substantial economic impact on a cattle operation. This paper outlines the pathogenesis, prevalence, economic impact and diagnosis of trichomoniasis in cattle, as well as reviewing common guidelines for the prevention and control of trichomoniasis.

Résumé

L'efficacité reproductive est l'un des principaux facteurs dont dépend la réussite économique d'un élevage de veaux d'embouche (atelier vache-veau). La trichomonase bovine, aussi appelée trichomonose, est une maladie vénérienne causée par le protozoaire *Tritrichomonas fœtus,* qui nuit à la reproduction d'un troupeau et réduit ainsi substantiellement la rentabilité de l'élevage. Cet article décrit la pathogenèse, la prévalence, l'impact économique et le diagnostic de la trichomonase des bovins, tout en rappelant les directives courantes de prévention contre cette maladie.

Introduction

Trichomoniasis is a major cause of fetal wastage that results in substantial economic losses wherever natural breeding conditions exist. Infected bulls are often asymptomatic carriers of *Tritrichomonas foetus* (*T. foetus*); however, they are capable of transmitting the organism to a cow or heifer during coitus. Infections in cows and heifers can result in early embryonic death, abortion, pyometra, fetal maceration, or infertility,[15,23,40,44] negatively impacting the economic success of a cattle operation. The economic losses associated with trichomoniasis are due to three factors: 1) reduced calf crop due to early embryonic loss or abortion; 2) reduced weaning weight due to delayed conception; and 3) culling and replacement of infected cattle. Since no legal treatment exists in the United States (US), veterinarians and cattle producers must focus on preventive management and other control measures. Understanding the pathogenesis, prevalence, economic impact and diagnosis of trichomoniasis will assist with implementation of appropriate prevention and control programs.

Pathogenesis and Clinical Signs

In bulls, *T. foetus* localizes in the smegma of the epithelial lining of the penis, prepuce and distal urethra.[43] *Tritrichomonas foetus* causes no penile or preputial lesions and does not affect semen quality or libido.[3,43] An infected bull, therefore, acts only as an asymptomatic carrier, and rarely clears the infection regardless of time. Deep preputial and penile epithelial crypts provide the appropriate microaerophilic environment required for establishment of chronic infections.[7,11,33,43] A common belief is that *T. foetus* infections in young bulls (less than 3-4 years of age) tend to be transient. However, any bull exposed to *T. foetus* in a natural breeding situation is capable of becoming chronically infected, regardless of age.

Tritrichomonas 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,[6] 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.[51] A small percentage of cows will not abort until the second or even third trimesters, and an even smaller number of cows (less than 1%) will maintain an infection through a normal gestation and deliver a live calf.[54] The few cows that maintain a *T. foetus* infection throughout gestation and into the next breeding season are very damaging since they represent a source of reinfection for the herd. Pyometra and abortion are often the first physical signs of trichomoniasis noticed in a herd, but these signs occur in less than 5% of infected animals.[3] Infertility due to embryonic death is the most economically damaging symptom 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).[6]

Unlike the bull, the cow typically mounts an effective immune response to *T. foetus*,[56] 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 days[44] or as long as 22 months.[2] Subsequent infections are cleared in about 20 days, indicating an anamnestic response.[46]

Immunity does not persist, however, and the anamnestic response is only significant if reinfection occurs within about 15 months of the primary infection.[15,43] 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.

Prevalence

Several estimates are available regarding prevalence of trichomoniasis in different regions of North America. In 1964, Johnson reported a 7.5% prevalence in western range bulls.[34] More recent studies from Florida,[1] Oklahoma[61] and California[8] found prevalence rates of 7.3, 7.8 and 4.1%, respectively. The Florida and Oklahoma studies sampled bulls from sale barns or abattoirs, while the California study sampled bulls from randomly selected herds. Rae *et al* conducted an even more recent epidemiological survey of randomly selected natural service beef herds in Florida between 1997 and 1999, and reported a 6% prevalence of *T. foetus*-infected bulls.[49] Riley *et al* also reported a 6% prevalence in bulls in Saskatchewan, Canada.[50] In other parts of the world, Erasmus *et al* reported a 7% prevalence in the North Western Cape Province, Western Transvaal and the Orange Free State in South Africa.[20]

Economic Impact

The economic impact of trichomoniasis is due to: 1) reduced calf crop from early embryonic loss or abortion; 2) reduced weaning weight due to delayed conception; and 3) culling and replacement of infected cattle. Rae developed a computer simulation model to study the impact of trichomoniasis on a cow-calf producer's profitability.[47] The model estimated a 14 to 50% reduction in annual calf crop if *T. foetus* infections were present in 20 to 40% of the bull population, and the net return per cow exposed to an infected bull decreased by 5 to 35%.[47] The economic impact of trichomoniasis can be so devastating that several western states in the US consider trichomoniasis a reportable disease and require bull testing prior to sale, prior to transport into the state, or before the use of public land.

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.[10,16,21,30,58] 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,[14,31,39] 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.[17] Therefore, research has recently focused on molecular-based assays to accurately differentiate *T. foetus*[21,22,32,50] from other trichomonads. Given the lack of legal therapy for bulls infected with *T. foetus*, 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%.[45] 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.[28,42]

Sampling techniques in the male

Several sampling techniques are utilized for obtaining diagnostic specimens in the bull including: 1) a swab technique;[41] 2) a dry pipette technique;[46,53] 3) a wet pipette technique;[36] and 4) the douche technique.[36] 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.[24] The swab technique is therefore rarely used in the US. The dry pipette technique is one of the most common sampling methods in the US, while the douche method is the preferred technique in Europe.[53] Schönmann *et al*

reported that the two methods are not statistically different.[53]

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.[25]

Sampling techniques 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.[7,36,46] An infusion pipette is used to aspirate cervicovaginal mucus (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 pyometra. Either sample is then examined directly or placed into appropriate culture medium. Culturing *T. foetus* from cervicovaginal mucus has a reported sensitivity of 58 to 75%.[55] Samples can also be evaluated with appropriate molecular-based assays.

In vitro *culture*

Direct microscopic examination of specimens for *T. foetus* is diagnostic, but a far more sensitive method for the detection of *T. foetus* is *in vitro* culture of preputial smegma or CVM in a selective nutrient medium for up to a week.[35,55,59] *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.[10,13,16] Alternatively, samples may be submitted directly for molecular-based evaluation.

	Result	Sensitivity (in series)
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.[25]

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[a] *Tritrichomonas 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 US. Both culture systems are fairly equal in sensitivity.[4,7,53,57] However, the InPouch™ TF is somewhat more convenient than Diamond's medium.[12] 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.[10,16,21,30,58] 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

One of the complicating factors associated with bovine trichomoniasis is that there are currently no effective treatments with US Food and Drug Administration approval.[46] Historically, the most successful treatment for bulls with trichomoniasis involved systemic treatment with nitromidazole derivatives.[5,19,26,55] However, the use of nitromidazole derivatives is now illegal in food-producing animals in the US because of their mutagenic and carcinogenic properties, and no alternative treatments are available. 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, ev-

ery cattle operation should focus on preventing the introduction of *T. foetus*.

Recommended practices to prevent 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.[27]
2) Utilize artificial insemination when possible.[46]
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.[54]
6) Test bulls for *T. foetus* at least once before introducing them into a new herd.[46] The test should be performed after two weeks of sexual rest. Ideally, a bull should have three negative cultures at weekly intervals.
7) Maintain as young a bull battery as possible. Older bulls are considered more likely to develop chronic *T. foetus* infections.[43,48] However, any bull exposed to *T. foetus* in a natural breeding situation is capable of becoming chronically infected, regardless of age.
8) 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.[36]
9) Consider immunization against *T. foetus* in high-risk herds.

Recommendation for control of trichomoniasis in an infected herd incudes:

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.[43,48] 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.[46] 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.[46]
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.[37]
7) Culture all pyometras diagnosed in cows or heifers during pregnancy examinations.
8) Submit all aborted fetuses and placental tissue to a diagnostic laboratory.
9) 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.[9,18,29,38,52,60] TrichGuard®[b] and TrichGuard® V5L[c] 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.

Endnotes

[a] InPouch™ TF *Tritrichomonas foetus* culture pouch – BioMed Diagnostics, White City, OR

[b] TrichGuard® – Fort Dodge Animal Health, Fort Dodge, IA

[c] TrichGuard® V5L – Fort Dodge Animal Health, Fort Dodge, IA

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