

Trichomoniasis in Cattle

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Bovine trichomoniasis is a sexually transmitted disease caused by the extracellular protozoa *Tritrichomonas foetus*, an obligate parasite of the reproductive tract of cattle. Infected bulls are often asymptomatic carriers of *T. foetus*. However, these infected bulls are capable of transmitting the organism to a cow during coitus. Infections in cows cause endometritis, cervicitis, vaginitis which may result in early embryonic death, abortion, pyometra, fetal maceration, or infertility. The major economic losses associated with *T. foetus* are due to: 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. Due to the inability to use efficacious drugs, such as the nitromidazoles, for control and prevention of *T. foetus* infections in food animals, most control efforts have targeted identification and elimination of positive bulls, systemic immunization of cows and bulls, and management strategies to prevent introduction of the organism into the herd. This paper will review trichomoniasis in cattle and discuss pathogenesis of disease, transmission, consequences of infection, immunity, diagnostic techniques, and control and prevention strategies.

Introduction

Bovine trichomoniasis is a sexually transmitted disease caused by the extracellular protozoa *Tritrichomonas foetus*, an obligate parasite of the reproductive tract of the cow and the folds on the mucosal surfaces of the bull's penis and prepuce. Infected bulls are often asymptomatic carriers of *T. foetus*. However, these infected bulls are capable of transmitting the organism to a cow during coitus.¹ Infections in cows cause endometritis, cervicitis, vaginitis which may result in early embryonic death,

abortion, pyometra, fetal maceration, or infertility.¹ The major economic losses associated with *T. foetus* are due to: 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. Due to the inability to use efficacious drugs, such as the nitromidazoles, for control and prevention of *T. foetus* infections in food animals, most control efforts have targeted identification and elimination of positive bulls, systemic immunization of cows and bulls, and management strategies to prevent introduction of the organism into the herd.

Pathogenesis in the Female

Life Cycle

The life cycle of *T. foetus* is thought involve two forms 1) a tear-shaped trophozoite form and 2) a pseudocyst form. The trophozoite is 10-25µm long and possesses three posterior flagella, one anterior flagellum and an undulating membrane. Trophozoite multiply asexually through binary fission.² Pseudocysts usually appear as a result of unfavorable conditions; although, a small percentage of pseudocysts exist under normal conditions.³ Pseudocysts occur when *T. foetus* trophozoites round up and internalize their flagella in response to assorted stimuli.³⁻⁵ The pseudocyst form lacks a protective cyst wall and does not represent a true cyst form.⁴

Trophozoites of *T. foetus* are transmitted between cows and bulls during coitus and remain in the genito-urinary tract where they multiply by longitudinal binary fission. Under stressful conditions trophozoites will internalize their flagella and replication of the nuclei and other cellular structures will occur, resulting in a multinucleated pseudocyst form. When conditions become desirable once more, mononucleate trophozoites will bud from the pseudocyst. In bulls, infections are usually chronic and asymptomatic and often persist for the life of the animal. Infected cows will initially experience vaginitis

which may or may not resolve spontaneously. In some cases, endometritis can occur resulting in complete sterility. *Tritrichomonas* infections may also result in fetal loss during pregnancy.⁶

Studies have revealed that pseudocyst formation and reversal can be rapidly and simply effected by certain cooling and warming patterns.⁴ However, the induction of pseudocysts by chemicals, dependent of exposure time and concentration, can lead to an irreversible process that leads to the death of the cells.⁷ Historically, there has been some uncertainty about whether pseudocysts represent a normal or infective form rather than a degenerative form. More recent research indicates that *T. foetus* is easily stimulated into the pseudocyst form and that these immotile pseudocysts are able to proceed with the process of adhesion to the vaginal epithelial cells.⁵ In addition, it has been demonstrated that the pseudocysts are more cytotoxic when in contact with host cells when compared to trophozoites.⁸

Transmission

Cows become infected with *T. foetus* primarily through coital exposure with an infected bull. Subsequently, a mild vaginitis occurs that may go undetected. The organism gains entry into the uterine lumen via the cervix during estrus. Colonization of the entire reproductive tract with *T. foetus* occurs within 1 to 2 weeks.⁹ Although, contaminated semen or contaminated insemination equipment may also be minor sources of infection.¹ Penetration of the vagina is seemingly necessary because swabbing the vulvar area with high numbers of organisms does not result in vaginal or uterine infection.¹⁰ Infected cows conceive but infection causes endometritis, cervicitis, or vaginitis which results in death of the conceptus within the first half of gestation, abortion, pyometra, fetal maceration, or infertility.¹ These infected cows usually remain infertile for a period of 2 to 6 months. In heifers, the duration of infection

is reported to be as short as 95 days¹¹ or as long as 22 months.¹² *Tritrichomonas foetus* has been detected in the reproductive tract for 13 to 28 weeks after experimental infection in heifers.¹³

Consequences of Infection

T. foetus organisms arrive in the female reproductive tract concurrently with spermatozoa. However in most cases, fertilization occurs in spite of the presence of the pathogen. In vitro studies have demonstrated that fertilization and early embryonic development to the hatching stage (8-10 days) are not significantly affected by simultaneous culture with *T. foetus*.¹⁴ Conceptus deaths most commonly occur between 50-70 days of gestation. Therefore, the majority of pregnancy loss is during the fetal period (>42 days of gestation). Although unusual, occasional abortions can occur of fetuses greater than four months of gestation.

Most producers do not recognize a problem in the early breeding season as conception occurs normally. The conceptus in most infected cows typically survives long enough to release sufficient interferon tau to prevent the prostaglandin $F_{2\alpha}$ -mediated lysis of the corpus luteum. Fetal death in infected cows occurs between 7 to 10 weeks of gestation. Death of the conceptus during the early stages of pregnancy results in a prolonged interestrus interval.^{9,15} Due to abortions and subsequent immunity, the distribution of pregnancies is unusually skewed with a higher proportion of pregnancies conceived towards the end of the breeding season. Although in many progressively managed herds with a limited breeding season, the bulls may no longer be available by the time the cow aborts and clears the infection. Therefore, *T. foetus* infection in a herd may go unnoticed until the time of pregnancy diagnosis when a high percentage of females are diagnosed not pregnant. Pyometra, along with abortions, may be the first physical signs of *T. foetus* infection in a herd, but are thought to occur in

less than 5% of infected cows.¹⁶ Pyometra results as the corpus luteum of pregnancy is maintained with a large purulent response which may cause damage to the uterine endometrium.¹⁷

Most infected cows will clear the organism and develop short-lived immunity of 6 months to one year. However, carrier cows do occur and are capable of spreading the protozoa. In the case of carrier cows, a very small percentage of cows (<1%) in infected herds have been shown to remain infected throughout pregnancy and into the following breeding season. Thus, the carrier cow has the potential to be quite devastating to control efforts and emphasizes that control programs must focus on the entire herd, not just the bull.⁹

Pathologic changes have been reported in several late-term, *T. foetus* aborted fetuses.¹⁸ The placentas had focal or diffuse invasion of the chorionic stroma by *T. foetus* as seen on hematoxylin and eosin (HE) stained sections of placentas. There was also evidence of a moderate inflammatory cell infiltrate comprised mostly of mononuclear cells. Six of eleven fetuses that were examined had bronchopneumonia with identifiable trichomonads in the airways. Another examination of late term abortions associated with *T. foetus* described a necrotizing enteritis and pyogranulomatous bronchopneumonia with tissue invasion by trichomonads. The exact mechanism that leads to the death of the conceptus is not fully understood. Although, cytotoxic and hemolytic effects by *T. foetus* on mammalian cells have been described.¹⁹

The preputial cavity of the bull provides an ideal environment for *T. foetus* as the organism localizes in the preputial smegma of the epithelium of the bull's penis and prepuce. The organism does not penetrate the epithelium and does not cause any observable gross pathology or affect semen quality or libido. Histological changes are subtle at first with an increase in the number of neutrophils in the nonkeratinized, stratified squamous epithelium of the glans penis and preputial epithelium followed

by an infiltration of lymphocytes and plasma cells penetrating into the intraepithelial area which coalesce in the subepithelium to form lymphoid nodules.²⁰

The duration of infection with *T. foetus* for bulls is not clearly understood. There are two theories regarding this debate: 1) transient infection and 2) chronic carrier state. Bulls with the chronic carrier infection of *T. foetus* rarely clear the infection regardless of time. The pathophysiology of infection regarding the carrier state in mature bulls is not fully understood. *T. foetus* infections in bulls less than 3-4 years of age are more likely to have a transient infection. Younger bulls may not efficiently transmit the organism to a noninfected cow unless the sexual contact occurs within minutes to days of breeding an infected cow. Thus, transmission of *t. foetus* by a young bull is thought to be more passive, mechanical transmission as compared to transmission in older, chronically infected bulls. Nonetheless, any bull exposed to a *T. foetus* infected cow as a result of natural breeding is capable of becoming chronically infected, regardless of age.

Immunity

In the female, *T. foetus* induces inflammation of the mucosa of the vagina, the cervix, the endometrium and the oviductal mucosa. In the first one to two weeks post infection, neutrophils and eosinophils predominate; however, this is followed by a moderate to severe mononuclear infiltration of lymphocytes and plasma cells. Subepithelial and periglandular lymphoid nodules resembling lymphoid follicles begin to develop at almost six weeks post infection. In addition, there is also an apparent degranulation of mast cells between six to nine weeks post infection.²⁰

T. foetus specific IgA and IgG₁ antibodies are detectable in uterine and vaginal secretions by the fifth to sixth week post infection. The IgA antibodies do not kill the organisms but may be responsible for immobilization and agglutination of parasites as well as preventing adhesion of the organisms to the mucosal surfaces. The IgG₁ antibodies are presumed to facilitate complement mediated lysis of the

parasites as well as opsonization and enhanced phagocytic killing by neutrophils or macrophages.

Immunity following natural infection and clearance of *T. foetus* is short-lived with females becoming susceptible within a year, in time for the following breeding season. Because *T. foetus* is an extracellular pathogen, the immune response from the host is predominately humoral and the result of the short-lived immunity. The uterine mucosal inflammation that is seen with infection may allow systemically derived IgG and complement to gain access to the lumen of the uterus and, thus, clear the organism. A relative lack of IgG from the vagina or possibly blocking of IgG effects by vaginal IgA binding of organisms may help explain the carrier state that can be seen in infected herds.²⁰

Although specific immunoglobulins have been detected in small amounts in preputial smegma by some researchers, there seems to be no effective acquired immunity to *T. foetus* in the mature bull.

Diagnosis

The comparison of diagnostic assays for detection of *T. foetus* infections has primarily focused on the bull. Collection of *T. foetus* samples from bulls involves recovering the organism from the preputial cavity of the bull. Several techniques have been described for collection of diagnostic specimens in the bull and include a dry pipette technique, a wet pipette technique a douche technique and a swab technique. While the douche method is preferred in Europe, the dry pipette technique is most commonly used in the United States. Regardless of which technique is used, it is generally recommended that bulls be given 2 weeks of sexual rest prior to sample collection in order to allow accumulation of the organism on the bull's penis and prepuce and a greater chance of recovery.

Isolation of *T. foetus* from the female is reported to be less sensitive when compared with techniques used for bulls.^{21,22} In one study, the InPouch™ TF system (BioMed Diagnostics, Inc; White City, OR) was more effective than Diamond's medium (88% versus 68% in detecting heifers that had

been experimentally infected with *T. foetus*.²³ The accuracy of prevalence in the cow most likely depends on the timing of sampling relative to exposure. The immune response in females begins to eliminate the infection within 8 to 10 weeks after exposure in unvaccinated females.¹³ Therefore, cultures from females are best performed before the infection is possibly eliminated by the immune response.²³

Sample handling is also crucial for accurate detection of *T. foetus*. When evaluating temperature and media type it has been found that when laboratory or field isolates were cultured in Diamond's medium or InPouch™ TF, all cultures were positive for *T. foetus* when maintained for up to 4 days at either 22° or 37°C. However, samples maintained at 4°C or less resulted in inconsistent sensitivity.²⁴ It is important to remember that time, temperature, type of isolate, and type of media all have an effect on the sensitivity of *T. foetus* culture.

Microscopic evaluation of cultured organisms is not sufficient to differentiate *T. foetus* from nonpathogenic intestinal or coprophilic trichomonads (*Pentatrichomonas hominis*, *Simplicimonas moskowitzi*, *Tetratrichomonas* spp., etc).²⁵ Therefore, several conventional and real-time polymerase chain reaction (PCR) assays have been developed for the definitive diagnosis of trichomoniasis, and this methodology has demonstrated some advantages over culture.²⁵ However, accurate PCR results are directly related to the quality of the sample, which can be affected by transport condition parameters such as temperature and time of transport to the laboratory. There have been a number of issues that have limited the sensitivity of various conventional PCR assays for the detection of *T. foetus*. These problems include DNA degradation, accumulation of inhibitory compounds, sample contamination, and unexpected amplification products.²⁶ One study demonstrated a decrease in sensitivity of PCR testing with samples that were stored for 5 days or more. However, PCR was in 100% agreement with culture as long as the PCR was performed within 24 hours of the sample being submitted.²⁶

A more recent study evaluated the effect of different simulated transport conditions on samples containing *T. foetus* for the diagnosis of trichomoniasis using culture and quantitative PCR (qPCR).²⁵ This study demonstrated that transport temperatures of 4–20°C for 1–3 days before culture reduced or temporarily inhibited parasite replication but maintained viability. Samples tested by either culture or qPCR would have been expected to give positive results. However, diagnosis of trichomonads by both methods was negatively affected when specimens were maintained at transport temperatures of 42°C for 24 hours or more. This study emphasizes the importance of ensuring that clinical samples arrive to the diagnostic laboratory within 24–48 hours and of avoiding temperature transport conditions above 37°C in order to achieve an accurate diagnosis of *T. foetus*. The effects of high incubation temperatures on culture and real-time PCR for *T. foetus* have also been evaluated following inoculation into the InPouch™ TF system.²⁷ This study showed that *T. foetus* was detectable microscopically in inoculated pouches incubated at 37°C regardless of exposure time (1, 3, 6 and 24 hours), whereas those samples incubated at 46.1 °C detected *T. foetus* only after 1 and 3 hours of incubation. *T. foetus* was detected in samples incubated at 54.4°C after only 1 hour. Testing using real-time PCR for all inoculated media samples (37°C, 46.1°C, and 54.4°C at 1, 3, 6 and 24 hours) produced positive results for all inoculated media samples. This study suggests that samples collected for culture alone should be protected from high temperatures.

Prevention and Control

One complicating factor with bovine trichomoniasis in the United States is the lack of effective treatments with U.S. Food and Drug Administration approval. Historically, the most successful treatment for bulls with trichomoniasis was systemic treatment with nitromidazole derivatives.²⁸ Currently, the use of nitromidazole derivatives is illegal in food-producing animals in the U.S., and no

effective alternative treatments are available. The lack of effective, approved therapies for bovine trichomoniasis emphasizes the need for appropriate preventive and control measures. Prevention of trichomoniasis includes the following recommendations: 1) avoid movement of animals (co-grazing, leasing of bulls, good fences); 2) utilize artificial insemination, if possible; 3) use a defined breeding season and cull all non-pregnant females after the breeding season; 4) purchase virgin bulls and heifers as replacements; 5) test all bulls for *T. foetus* prior to introduction into the herd and maintain a young population of bulls; and 6) breed purchased cows and heifers in a separate herd.⁹

Once *T. foetus* has been confirmed in a herd, there are additional measures that should be considered in order to “clean up” the herd. These measures include 1) testing and culling all infected bulls and purchasing *T. foetus* negative bulls; 2) intense management of bulls so that smaller breeding units are used and bulls are bred to the same cattle until trichomoniasis is under control; 3) create high and low risk herds; and 4) vaccinate all herd females with an approved *T. foetus* vaccine.⁹ Vaccination is an important aspect of any control program as it has been shown to reduce pregnancy wastage associated with *T. foetus* infection in cattle herds. Currently, TrichGuard® (Boehringer Ingelheim Vetmedica, Inc.) is the only commercially available vaccine licensed by the USDA for the control of trichomoniasis in the United States. TrichGuard® is a proprietary vaccine that is a Freund adjuvant killed *T. foetus*-derived vaccine that requires two doses subcutaneous injections administered 2 to 4 weeks apart with the last injection to be given 4 weeks prior to the breeding season.⁹ One study compared pregnancy and calving rates between beef heifers vaccinated with TrichGuard® and control heifers after heifers were exposed to *T. foetus* infected bulls and intravaginally inoculated with a large number (10 million) of *T. foetus* organisms.²⁹ At calving twice as many vaccinated heifers calved when compared to control heifers (61% versus 31%). Thus, the vaccine appeared to offer at least some protection against *T. foetus*. More recent studies have confirmed these findings, as well.³⁰ In addition, Palomares, et al. concluded that vaccination of heifers with TrichGuard® significantly increased the levels of IgG

antibodies to the *T. foetus* surface antigen in serum, vaginal secretions, and uterine fluid; these antibody levels remained elevated through days 43, 75, and 182, respectively.³¹

Conclusion

Trichomoniasis can be an economically devastating infection in cattle herd with losses due to reduced calf crop due to early embryonic loss or abortion, reduced weaning weight due to delayed conception, and culling and replacement of infected cattle. Carrier females and concerns with diagnostic sampling and testing have made the control of trichomoniasis in cattle even more complex. Control and prevention of *T. foetus* infections in cattle must focus on identification and elimination of positive cows and bulls, systemic immunization of cows and bulls, and management strategies to prevent introduction of the organism into the herd.

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