

Update on Trichomoniasis

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Abstract

In recent years diagnostic surveillance for bovine trichomoniasis has increased since most states require testing prior to interstate movement of non-virgin bulls. However, bovine trichomoniasis continues to be an economically significant disease, particularly in beef cattle, in much of the United States. The disease produces no clinical signs in bulls but can have devastating reproductive consequences on females resulting in reduced calf crops and extended calving seasons. The organism is efficiently transmitted via the venereal route. Although prevalence may be highest in older bulls it has been documented to occur in non-virgin bulls of any age. Bulls are believed to be persistently infected but females develop short lived immunity and naturally clear the infection within 3-22 months. Diagnosis is made by culture in selective media and/or by PCR identification of the organism in smegma samples collected by preputial scraping.

Samples of reproductive or fetal fluids obtained from females can also be tested. Readers should consult their state veterinary diagnostic labs for instructions on sample preparation and shipment.

Control of trichomoniasis is primarily through testing and culling of positive bulls. There are no approved, effective treatments available. However, vaccination of females will, in most cases, improve reproductive indices in infected herds.

Key words: cattle, trichomoniasis, bull

Introduction

Bovine trichomoniasis is a venereal disease of naturally bred cattle due to infection with the protozoan *Tritrichomonas foetus* (*T. foetus*). Trichomoniasis is a significant cause of economic loss to the cattle industry largely due to fetal wastage and culling of infected bulls. The disease is inapparent in bulls but is associated with repeat breeding, extended calving, embryonic death and occasional late-term abortion or mummified fetus, and a high percentage of open cows at time of pregnancy evaluation (mean of 18% less than herds with no infected bulls).⁷ Bulls, however, are very efficient spreaders of this disease to non-infected female cattle. A single mating with an infected bull resulted in 95% infections among susceptible nulliparous cows.¹⁹ Economic losses to the U.S. beef industry results from reduced calving rates, lower weaning weights and increased culling. A 2013 study by David Anderson at Texas A&M University estimated losses

to Texas producers of \$95 million annually.² There are no approved treatments for infected cattle. Biovigilance (closed herd, fencing) and appropriate testing and culling of infected bulls are essential to minimize losses due to this disease.³

The Organism

Isolates of *T. foetus* are identified on the base of morphologic features: small flagellates 10–25 μm long and 3–15 μm wide, with three free anterior flagella and a recurrent one forming a well-developed undulating membrane that runs almost the entire length of one lateral edge of the protozoan.¹² *T. foetus* has an affinity for cattle but has been isolated from the gastrointestinal tract of swine and cats. Although the same species of *T. foetus* infects swine and cats these isolates are considered to be distinct serotypes and unlikely to cause disease in cattle.¹¹ *T. foetus* is not considered to be zoonotic but severe infections have occurred in a few immunocompromised individuals. Other trichomonads, considered non-pathogenic in cattle, include *Tetratrichonaona* spp and *Pentatrichomonas homini*.¹⁰ These species have both been isolated from the prepuce of bulls which can result in misdiagnosis based on culture results.¹¹ Avoiding sample contamination by fecal trichomonads is achieved by clipping the preputial tuft of hair and cleaning the preputial orifice. However, PCR readily differentiates trichomonad species.

The Disease

T. foetus is transmitted primarily by the venereal route from infected bulls to cows and heifers during coitus. Bull to bull transmission may occur but rarely. Iatrogenic transmission may occur via operator's gloves during genital examination. Infected bulls are largely asymptomatic but maintain semen quality and libido. A preputial discharge associated with small nodules on the preputial and penile membranes may occur shortly after infection. Nevertheless, chronically infected bulls usually develop no gross lesions although they carry a small number of the organisms in the prepuce with some concentration in the fornix and around the glans penis. They remain asymptomatic carriers of infection for years and, possibly, for life. The organism colonizes the stratified squamous epithelial surfaces of the glans penis, prepuce and distal urethra. Since the infection is only superficial, an immune response is not induced and the organisms persist indefinitely. Female cattle typically clear the infection within a few months and develop a partial but short-lived immunity.

Some females may remain infected for up to 22 months which may result in the disease being perpetuated in a herd even when all infected bulls are culled. The uninfected bull may acquire infection from a chronic female carrier then spread the disease through subsequent mating.

In the past, it was thought that young bulls (<3 years) could only become transiently infected as their preputial crypts (necessary to provide a microaerophilic environment) are not well developed. It was also thought that Brahma-influenced breeds were more susceptible to chronic infection due to the depth of their preputial crypts. Recently, it has been demonstrated that bulls of any age or breed can become chronically infected, but it still remains more likely to occur in the older bulls. As an example, in a beef herd investigation it was found that only three of 19 bulls 1-2 years of age were infected in contrast to 12 of 13 bulls aged 3-7 years. The effect of *T. foetus* var. *brisbane* infection on calf production by Hereford cows was determined. The mean number of calves produced by cows that were kept continuously with bulls infected with *T. foetus* for 3 years was 17.6% less than the mean number produced by cows kept with a non-infected bull. Losses in production due to trichomoniasis occurred each year but were greatest in the first 2 years in cows experiencing infection for the first time.⁸ Similar patterns of infection have been observed worldwide.

Once a female becomes infected, the organism adheres to the vaginal epithelium then colonizes and spreads to the cervix, uterus, and uterine tubes.³ The presence of the organism in the female reproductive tract does not impair fertilization and initial embryonic development. Most pregnancy loss occurred after 50-70 days. This is also the time of peak luminal IgG₁ and IgA antibody responses in the female reproductive tract. Uterine clearance of trichomonads begins before vaginal clearance, but in any case, by 12-18 weeks after infection, most cows are free of the organism and regain their fertility. Immunity is short-lived however, and convalescent cows may be reinfected in the following breeding season. A small proportion of exposed cows, perhaps 5%, develop a post-coital pyometra. This infection results in vaginitis and endometritis that culminates in fetal death. In most cases, these early pregnancy losses are unobserved and it is not until later when the cow is found to be non-pregnant or was bred late in the breeding season.³ Most infected females are able to mount an immune response and clear the infection within weeks to months although occasionally, females will harbor the organism throughout gestation and deliver a live calf.²¹ Visible abortions are traditionally associated with trichomoniasis but, in fact, these events are rare, occurring in less than 5% of infected females.¹

Diagnosis

Routine screening for trichomonads typically is directed at the bull battery. The standard approach has been to test after seven days of sexual rest by the dry

pipette method.²⁰ The sexual rest period is to preclude a false negative from mechanical reduction in trichomonads during breeding. The technique involves placing a sterile infusion pipette into the preputial cavity and attaching a 12 cc syringe. The pipette is moved briskly back and forth and smegma is aspirated into the pipette. The smegma is then inoculated into a commercially available trichomoniasis transport/culture device^a and placed upright in a microbiological incubator set at body temperature.⁵ Alternatively, the smegma can be placed in a special transit tube^b. Once the sample is obtained there are multiple alternative testing procedures. When culture and visual observation is employed the culture is examined microscopically daily for seven days to determine presence/absence of trichomonad organisms. The organism is recognized by its typical size, morphology, and characteristic jerky motion.²² If organisms are found it is recommended that the diagnosis be confirmed by PCR. PCR can unequivocally distinguish *T. foetus* from other non-pathogenic trichomonads and thus avoid a false-positive diagnosis.¹⁴ To improve sensitivity, it is recommended that three consecutive cultures be obtained at weekly intervals in order to be 99% certain the bull is not infected.⁶ More recently most states have recognized a single PCR test as 'official' and adequate for regulatory purposes but a few false negatives can occur especially if samples are pooled. A few years ago, one of our residents conducted a study to determine the best sampling site and laboratory methods to diagnose trichomoniasis.¹⁷ It was determined that it makes no difference whether the glans penis or preputial fornix was sampled. However, technically, obtaining samples from the preputial fornix was judged to be easier. The commercially available PCR test^c compared favorably with traditional culture methods for detection of trichomonads.¹⁶ It was found there was 100% agreement between TaqMan™ PCR results when a single sample was incubated for 24 h prior to amplification and three cultures subjected to InPouch™ TF culturing at seven-day intervals.¹⁷ Pooling of smegma samples is permitted in some states. Real time PCR combined with culture results may be recommended in the future for diagnosis of trichomoniasis.¹⁸ For example, in Oregon, the state veterinary diagnostic laboratory accepts either of two methods of sample handling prior to PCR analysis as described below.

Method 1: After sample collection, ship samples in transit tube or InPouch™^a overnight so as to arrive at the lab within 48 hrs.

Do not ship on ice - ship at room temperature.

Method 2: If you are not able to ship samples to lab within 48 hours (e.g. due to a holiday, weekends, or weather delays), then freeze samples within 48 hours after collection. Frozen samples can be stored at 20°C. When ready to ship, ship samples overnight on ice in a styrofoam container so they can arrive at the lab below 20°C.

Incubation is no longer required prior to shipping for either the transit tube or InPouch™^a. Incubated samples (24 to 48 hours at 37°C) will still be eligible for official testing but is no longer necessary for official testing requirements.

Samples received outside these 2 methods can still be run as a diagnostic result only and are not considered an official test. Results will not be valid for movement, sale or other regulatory purposes. Remember, do not use expired pouches or transit tubes as they may be rejected by the lab and cannot be used for official testing requirements.

It is important to note that in Oregon as well as other states, the veterinarian collecting the samples must be certified by the state field veterinarian.

Veterinarians are encouraged to contact their respective state animal health officials to remain current on recommended procedures and regulations.

In the case of female cattle or aborted calves in which trichomoniasis is suspected, examination of vaginal aspirates (cervicovaginal mucus)⁴, uterine discharge, placental fluids, and fetal stomach contents can be useful. Samples may be evaluated by culture or PCR.

Control

Bull testing is the mainstay of control measures for trichomoniasis. The intensity of testing is dependent on herd history and regional likelihood of trichomoniasis. At the least, all purchased bulls should be tested before joining the herd provided trichomoniasis has not been diagnosed in the herd previously. In herds or areas where the disease is endemic all bulls should be tested prior to being turned out with females. All bulls should be tested annually if they have been comingled with animals from other herds as in public grazing areas or in grazing co-ops. Any positive bull should be sent immediately for slaughter since there is no legal effective treatment. In most states, trichomoniasis is a reportable disease. Strong evidence for a benefit to vaccinating bulls is lacking. However, in one study there was a tendency for bulls under 5.5 years of age to either resist infection or be cured by vaccination.⁹

Where the disease is endemic, female cattle should be vaccinated. The current vaccine^d will not prevent infection but will reduce the time it takes for the female to clear the infection and thereby reduce the risk of abortion.¹⁵ Immunization is obtained by injecting an initial dose followed by a booster two to four weeks later with the second dose should be given about four weeks prior to the breeding season. Annual revaccination, four weeks prior to the breeding season is recommended. A killed, whole cell commercially available was effective in reducing losses due to *T. foetus* infection in female cattle.¹³

Purchased cows and heifers should be kept in separate breeding herds for at least one season, especially if they originate from a high risk region. Avoiding the most potentially devastating risk factors including; lack of bull testing, and

mixing herds when grazing, along with culling positive bulls will reduce losses due to trichomoniasis.²³

Endnotes

^aInPouch™ TF, BioMed Diagnostics, White City, OR

^bTF-Transit Tube, BioMed Diagnostics, White City, OR

^cTaqMan™ qPCR, Applied Biosystems, Foster City, CA

^dTrichguard®, Boehringer Ingelheim, Duluth, GA

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