

Bovine trichomoniasis overview and testing

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Abstract

Bovine trichomoniasis, caused by *Tritrichomonas foetus*, is a venereal disease of cattle, resulting in infertility, pregnancy losses, abortion and delayed calving seasons. The disease has global distribution, with greater prevalence in areas in which natural service is the primary means of reproduction. When artificial insemination, with appropriate hygiene practices, is implemented, as well as aggressive testing and culling of positive bulls, control and eradication of the disease is possible. Additionally, minimizing co-mingling of animals with unknown status through herd additions or on public grazing lands will help decrease the likelihood of introduction into negative herds, and contribute significantly to the decrease in prevalence in the local region. Testing methods have improved over the last 100 years; however, proper sample collection and handling are still paramount to gaining true positive results in infected bulls.

Key words: trichomoniasis, *Tritrichomonas foetus*, infertility, abortion

Introduction

The causative agent of bovine trichomoniasis (*Tritrichomonas foetus*) is a motile protozoan that has nearly global distribution, with occurrences documented on every continent except Antarctica.^{1,2} The protozoan is named for its three anterior flagella, one posterior flagella, and undulating membrane.^{2,3} These anatomical structures give the organism its classic tumbling motility pattern.³ There are many trichomonads that exist in nature, infecting different species in different ways. For example, *Trichomonas vaginalis* is a human parasite and is transmitted through direct sexual contact similarly to bovine trichomoniasis.¹ By contrast, *Tritrichomonas blagburni*, a parasite found in domestic cats, once thought to be identical to *T. foetus*, has been suggested to be a distinct species, and is a cause of diarrhea in cats.^{4,5}

The impacts of *T. foetus* infection are well documented, and many quality literature reviews have been written on the subject.^{3,4,6} At the herd level, the astute producer may notice abnormal late returns to estrus after bull turn out or find cows with a post-breeding pyometra at early pregnancy diagnosis, but typically evidence of herd exposure is noted at the conclusion of the breeding season at pregnancy diagnosis. An unusually high percentage of open cows and a very “end-loaded” calving season are highly suggestive of trichomoniasis, and require investigation. In addition to infertility and subfertility, exposed cows are at a greater risk of abortion and the birth of weak calves.^{7,8}

Persistence in beef cattle

Infection in cows typically results in disease clearance and return to fertility, but this is not always the case. Some cows are able to remain asymptomatic carriers, and deliver live and healthy calves, giving no indication that they remain infected.^{7,8} These cows are then the reservoir host to infect otherwise uninfected bulls. Once a bull becomes infected, he is an asymptomatic carrier for life.¹ There are currently no approved methods to treat trichomoniasis in the bovine male.¹⁰ Young bulls

are more resistant to infection than are older bulls, which is an important feature in control and eradication programs.⁶

Regions in which trichomoniasis remains a challenge are those in which natural service is the predominant means of breeding, and in those regions in which there is a significant amount of comingling of herds for grazing purposes. Beef herds are at greater risk for introduction of *T. foetus* into the cow herd, and this risk increases from east to west on the North American continent. Based on the NAHMS Beef 2017 data, a lower percentage of operations in the eastern region of the United States tested bull additions that were 18 months of age or older or what were not virgin (39% vs 64% and 70% for the western and central regions, respectively). Similarly, only 10.5% of operations in the eastern region tested bulls for trichomoniasis as part of a routine bull breeding soundness evaluation (compared to 26.2% and 25.7% for the west and central regions, respectively).¹¹

Though trichomoniasis is generally considered a disease of natural service, there is a risk of transmission of *T. foetus* frozen semen is contaminated with *T. foetus*-contaminated seminal fluid.^{4,12}

The ability for *T. foetus* to evade the host immune system is theorized to be related to the organism’s capacity for internalizing and neutralizing IgG.³ The organism is not efficient at destroying IgA, and therefore this immunoglobulin plays an important role in decreasing the clinical severity of the disease, however IgG is still critical in the process of elimination of the parasite from the host. Because there are relatively low levels of antibodies in the bovine reproductive tract, *T. foetus* is able to populate this system and successfully evade the host immune response.⁴

Control and eradication

Successful control and eradication programs are multi-faceted endeavors that tackle the problem of trichomoniasis from many directions.^{1,6,12} Adequate and thorough testing and culling of positive bulls is a very significant component to understanding the prevalence of the disease, and is one of the most intuitive steps in a control and eradication program.

Though, as previously mentioned, artificial insemination, in cases of insufficient testing or hygiene practices, may be a potential route of transmission of the protozoan. However, the vast majority of frozen semen is free of this parasite, and the practice of estrus synchronization and artificial insemination can be a path to control or minimize the potential spread within a herd.^{1,6}

The current beef management practices in this country, due to labor requirements for synchronization and artificial insemination, the timing necessary for success of these programs, and the economic need for every beef cow to calve every year precludes the massive widespread adoption of artificial insemination across the beef industry. Therefore, natural service will always be a necessary component of beef cow reproduction. The use of virgin bulls or young bulls will also be an important strategy to include in the control and eradication program, as young bulls are less likely to become carriers than are older bulls.^{1,6,12}

Another important component of a successful program is herd biosecurity. Attempts should be made to minimize the likelihood of introducing *T. foetus* into the beef herd by various means. Careful scrutiny of herd additions through sourcing or pre-entry testing can be a valuable means of preventing introductions, as is the rearing of replacements within the herd. Through the use of assisted reproductive technologies, such as artificial insemination or embryo transfer, a small select group of cows could be identified as the “nucleus herd” in which replacements (males or females) could be generated. This strategy can allow for introduction of new genetics, while minimizing the risks of introducing many undesired pathogens. Co-mingling on public grazing lands can create a significant risk to biosecurity of the herd and should be critically evaluated to determine if the risk is worth the benefits. On private pastures, quality fencing that is in good repair and inspected and repaired (as needed) prior to cattle turnout will pay dividends in the long term as it effectively excludes maverick cattle from co-mingling with the operation’s resident herd.^{1,6,12}

Vaccination should also be considered as a management practice when control and eradication is the goal. Vaccination will not prevent infection; however, the severity and duration of the infection is decreased in females that have been properly vaccinated.⁹ This will ultimately decrease the impact on the herd, as a whole, in the face of an exposure to *T. foetus*. It is important to note that proper vaccination (timing, boosters, product storage/handling, etc.) is key to gain maximum effect and minimize the effect of the disease. Recently, a research group in Argentina has been working to identify new and novel vaccines and delivery systems that may have the potential for providing a similar level of immunity, but with a single intervaginal dose of vaccine, which may possibly eliminate the need for boosters.¹³

Bovine trichomoniasis is considered a reportable disease in many states and provinces throughout the world. The active engagement of regulatory medicine has made eradication a success story in many locales. Many European countries have been successful in decreasing incidence by aggressively implementing artificial insemination at a standard breeding strategy (i.e. Poland is free as of 1997).¹³ A recent report in Veterinary Research, published by Dr. Yao, indicates through testing and culling, Wyoming had successfully maintained zero positive test results in bulls sampled between 2017 and 2019.¹ Likewise, through regulation of testing and animal movement, Texas has experienced a decrease in prevalence of trichomoniasis in tested bulls (T. Hairgrove, DVM, PhD, DABVP, Department of Animal Science, Texas A&M University, email, September 9, 2022).

Potential opportunities for treatments

There are currently no labeled treatments for trichomoniasis in cattle.¹⁰ As previously discussed, cows typically will clear the infection after a period of infertility, with a few remaining as carriers in the herd. There has been some work looking into novel uses of compounds that have previously been shown to have efficacy against the protozoan that causes malaria (*Plasmodium falciparum*).¹⁰ In this work, there were 4 compounds identified that had lethal activity against *T. foetus* in vitro, one of which was nearly identical in chemical structure to a compound that has historically been used in human medicine, and currently has application of use as a mold inhibitor in feeds. More work needs to be done in this research area before a viable treatment option is developed, but these results are promising. In contrast to experimental therapeutics, Love, et al.,

explored the use of the antimicrobial drug metronidazole to treat trichomoniasis in bulls, as this drug has been used to effectively treat trichomoniasis in humans.¹⁵ However, this drug is banned for use in food animals, due to potential carcinogenic activity in animal studies.¹⁵ These studies, however, have come under scrutiny, as the conclusions may have not factored in the other side effects of metronidazole treatment as the cause of the increased cancer risk (i.e. increased body weight, increased lifespan), and instead supposed a direct effect.¹⁵ Many researchers, veterinarians, and cattle producers would appreciate a fresh look at metronidazole by the United States Food and Drug Administration, as a potential treatment for trichomoniasis in cattle.

Testing for *T. foetus* Testing bulls

Effective treatments would dramatically increase the likelihood of eradication and accelerate the process of eradication of trichomoniasis in cattle. However, in the absence of treatment, testing becomes an important component of eradication and control. The gold standard test for trichomoniasis has historically been culture. Unfortunately, culture is not without its challenges. The protozoan, *T. foetus*, is an anaerobe that does not tolerate over heating or over cooling well. For these reasons, sampling and test submission procedures become critical to the likelihood of a true positive result.⁶ The most common vehicle for culture is the Biomed InPouch TF test kit. A quality detailed description of collection and sample submission can be found at the Kansas State Veterinary Diagnostic website at <https://www.ksvdl.org/resources/videos/>.

When preparing to sample bulls for trichomoniasis culture, be sure to schedule the sampling and shipping to assure that the samples can arrive at the lab within 48 hours of sample collection, and that the samples will be protected from extreme heat or cold. Poor collection technique, temperature extremes, poor sample or sample kit handling, or delays in transit can have potentially negative impacts on the likelihood of obtaining a true positive result, and false negatives are common.⁴ For these reasons, it is often suggested, and sometimes required, that 3 negative cultures be obtained 1 week apart, before a bull can be confidently considered negative.

In contrast to culture, another testing modality available is *T. foetus* PCR. Given that PCR identifies the presence or absence of the DNA of the protozoan, and does not rely solely on live organisms for a positive test, there are some benefits to this test when compared to culture. The sample can sustain increased time in transit without compromising the quality of the sample. Additionally, PCR can be performed alone as an official test for animal movement, or can be used as a confirmatory test on a sample that is culture positive, to strengthen diagnosis. One of the drawbacks of PCR is the potential for false negatives if the sample is contaminated. Bacterial contamination may expend the nutrients of the media in the test kit, which will decrease the availability for adequate nutrients for the growth of the target protozoan, as well as the creation of an acidic environment within the test kit media, which has the potential to degrade the DNA of the *T. foetus* organisms, and result in a false negative test.⁸ Additionally, there have been reports of cross-reactions of other protozoal organisms that may be saprophytic or commensurate organisms in the rumen of cattle, leading to a false positive result.¹⁴ This necessitates appropriate hygiene practices. Prior to collection, the hairs of the sheath should be cut with clean scissors, if necessary, the prepuce should be flushed

with 60 mL of saline prior to sampling, and finally, a sterile and guarded insemination pipette attached to a 12 mL syringe should be used for collecting samples from bulls. These steps should be taken whether the collected sample will be cultured or be subjected to PCR testing.¹⁶

Though many states accept a single negative PCR test for interstate movement of bulls, there is some risk associated with this practice. Testing procedures themselves are fairly straightforward and a quality sample collection is not difficult to perform. Inaccuracies are more likely to arise with cases in which sample handling has been compromised in some way. The media required for culture is not exceptionally stable, and if it is not stored in a manner consistent with lab instructions, the trichomonads will not likely survive.⁴ In chute-side applications, the media must be protected before inoculation, as carefully as after sample collection, to avoid compromising its nutritive value, and to avoid putting smegma into a sample pouch that is either too hot or too cold. Packaging samples should be done consistent with federal regulations, and the samples should arrive at the lab within 48-96 hrs of collection.¹⁶ It has been suggested that samples for trichomoniasis culture should not be shipped on ice, as this creates an adverse environment for the organism.¹⁶ But, it is important to note that in the absence of a temperature-regulating device, the samples are at risk for temperature fluctuations in transit that can have the potential to compromise the sample and return a false negative result. Work by Carly Summarell, Tom Hairgrove and others at Texas A&M have suggested that shipping samples for PCR testing (specifically RT-qPCR) should be shipped with ice packs, provided there is a layer of insulation (i.e. paper towels) between the sample and the ice pack.⁸ This will allow for more consistent temperatures inside the shipping container, while avoiding direct contact of the sample with the extreme cold of the ice pack.

When testing bulls, it is recommended that testing should be conducted before the breeding season, as well as at the conclusion of the breeding season. This is especially important in cases in which cattle may be co-mingled with animals of unknown status. This double testing helps to prevent introduction of *T. foetus* into the herd, and a potential exposure can be identified in a much more rapid manner, allowing for a quicker implementation of appropriate interventions to minimize the potential future spread of the disease. Furthermore, there is evidence that bulls can have a significant variation in their parasite load over time, and a single test may not effectively capture a true positive (T. Hairgrove, DVM, PhD, DABVP, Department of Animal Science, Texas A&M University, email, September 9, 2022).

Testing cows and heifers

In the face of an active infection, testing females requires aspiration of cervical and uterine contents, and inoculating the sample media. However, in a non-clinical open female, testing sensitivity decreases and a true positive result is more difficult to obtain. In contrast to bulls, in which 3 negative cultures must be obtained one week apart, an open female requires 5 negative tests, 1 week apart, to increase the confidence interval to an acceptable level. This makes testing females the less efficient means of surveillance and for control and eradication purposes. Additionally, pregnant cows may be infected with the organism, but retrieval of the parasite from the distal parts of the gravid tract is not feasible, and sample collection from the vagina will result in a false negative test (T. Hairgrove, DVM, PhD, DABVP, Department of Animal Science, Texas A&M University, email, September 9, 2022). However, one cannot ignore

the contribution of asymptomatic carrier cows as host reservoirs of the disease. From a regulatory standpoint, some states are implementing animal movement requirements for females. In South Dakota, there are animal movement requirements for cows that are moved into the state, in an attempt to decrease the likelihood that a carrier cow could reintroduce the disease or disrupt the eradication efforts in place.¹⁹ Oklahoma and Kansas, as other examples, have established regulatory protocols for cows that have been exposed to an infected bull and may have the potential to have been exposed to *T. foetus* during the breeding season, to better control the movement of the disease within the state.^{20, 21}

Conclusion

Bovine trichomoniasis has been a disease challenge for cattle producers for well over 100 years. As technology advances, the prospects of global eradication of this disease increases, even in the face of not possessing availability of effective treatment regimes. Artificial insemination as an alternative breeding strategy to natural service, can be used to interrupt disease spread, whether it is implemented temporarily or permanently. Other strategies, such as maintaining good fences, avoidance of co-mingling animals of unknown status, aggressive testing and culling of positive bulls, and using only young bulls (<4 years of age) will also provide valuable components of an eradication program. Lastly, careful handling of open cows or those that abort as potential asymptomatic carriers can help to decrease the incidence of infection of negative bulls, and potentially accelerate eradication efforts.

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