Review of bovine trichomoniasis

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Abstract

Bovine trichomoniasis, caused by the protozoa *Tritrichomonas foetus*, results in reproductive failure and substantial financial losses. It was first reported in the US in 1932 and in the western beef herds in the US in 1958. Western herds, along with herds in certain areas of Florida, are considered the traditional range for this disease in the US. Since characteristics of the causative organism are well known, its control and even eradication are possible. Background information of *T. foetus* as it relates to etiology, pathogenesis, diagnosis, and control is provided.

Keywords: *Tritrichomonas foetus*, cattle, trichomoniasis

Introduction

Trichomonads were first reported as a cause of bovine infertility in France in 1888.[1] In the US, it was first noted in 1932 in Pennsylvania dairy cows[2] and in 1958 in western US beef herds.[3] Currently, *T. foetus* has been practically eliminated from intensively managed cattle populations around the world where the management includes limited commingling of cattle and artificial insemination is commonly practiced. However, it remains endemic in some herds in the western US managed under range conditions with natural service.[1]

Trichomoniasis exerts its most prominent impacts in a cattle operation through its negative effects on herd reproductive performance, resulting in fewer pregnant cows and consequently fewer calves for sale. Additional factors related to trichomoniasis that exert a negative influence on the profitability of a beef cattle operation include feed and other maintenance costs for nonproductive cows, replacement costs of *T. foetus*-infected bulls and nonproductive females, testing costs to control *T. foetus,* and reduced weaning weights due to late-born calves.[4,5]

It is difficult to estimate the prevalence of bovine trichomoniasis with any degree of confidence. Estimates in California[6] and Florida[7] had a herd-level prevalence of 15.8 and 28.8%, respectively, whereas bull-level prevalence was 4.1 and 6.0%, respectively. In Colorado and Nebraska abattoirs, only 0.172% of bulls' samples were culture positive for *T. foetus*.[8] Recent prevalence estimates of infected bulls in Alabama[9] and Tennessee[10] were 0.27 and 0.18%, respectively, with a prevalence estimate of 2.17% for Wyoming herds.[11]

Although the prevalence of bovine trichomoniasis is typically lower in nonendemic areas, its potential for substantial economic loss from this disease makes it a concern for the cattle industry. The following narrative summarizes the basics in bovine trichomoniasis regarding its etiology, pathogenesis, diagnosis, and control.

Etiology

Tritrichomonas foetus is a spindle- to pear-shaped single-cell protozoa with 3 anterior flagella. It has an undulating membrane along the length of its body containing an accessory filament at its margin and a single posterior flagellum. The organism ranges from 9 - 25 µm in length and 3 -15 µm in width, and replicates through asexual longitudinal binary fission.[12] Although the life cycle of this protozoa does not include a cyst form, it can develop into a pseudocyst.[13] The organism has an affinity for the bovine reproductive tract due to its limited oxygen concentrations, ample food supply in host's red and white blood cells, and bacteria. Hence it is considered an obligate parasite of the bovine reproductive tract.[12] In addition, *Tritrichomonas foetus* is capable of surviving temperatures used to store frozen semen for artificial insemination.[14]

Pathogenesis

Transmission

Natural transmission of *T. foetus* is considered strictly venereal and occurs during coitus.[15] The rate of natural transmission is high, with many naïve susceptible females becoming infected after a single exposure through coitus to an infected bull with

as few as 200 *T. foetus* organisms.[16] Bulls became chronically infected after 10^4 *T. foetus* organisms were experimentally inoculated into prepuce and some bulls became infected from inoculates containing as few as 10^2 organisms.[17]

Impact in the female

Tritrichomonas foetus can be isolated from the female bovine reproductive tract as early as 4 days after introduction.[18] However, it does not interfere with conception or maternal recognition of pregnancy[19] nor induce any macro- or microscopic lesions in the reproductive tract until 50 days of pregnancy, which are characterized by mild inflammatory changes and eventual fetal loss in a majority of the infected females up to 95 days after exposure.[20] Early fetal death and abortion are followed by early return to estrus in females, the most common clinical sign.

Fetal loss is followed by 2 - 6 months of infertility, as the immune system cleared the parasite from the reproductive tract.[19] This immune response led to a limited amnestic response to repeated *T. foetus* infection as demonstrated in multiple studies, with the longest estimated duration of partially protective immunity being < 15 months.[21]

With some notable exceptions (namely pyometra and chronic infection) complete clearance of *T. foetus* from the female reproductive tract typically occurs in 5 - 20 weeks.[18] Pyometra may be an earliest clinical sign of *T. foetus* infection in a cow herd;[22] in affected cows, purulent debris in the uterine lumen frequently had large numbers of *T. foetus*.[2] Cows were infective for as long as 300 days[23] or 22 months postbreeding.[24] Carrier cows had infection through normal pregnancy with *T. foetus* isolated up to 9 weeks[25] or 63 - 97 days[26] after delivery of an apparently normal calf.

Impact in the male

Tritrichomonas foetus is primarily an inhabitant of the superficial layers of the penile and preputial epithelium, but not routinely present in other locations of the male reproductive tract.[27,28] The absence of tissue invasion may be the reason for a limited immune response in T. foetus-infected bulls.[29]

Lack of pathological changes and failure of the immune response to eliminate *T. foetus* from the preputial cavity leads to chronic infection in older bulls. This relationship between age and *T. foetus* carrier bull status may be due to development of crypts in penile and preputial epithelium.[12] However, a more recent study disputes this longstanding dogma.[30] Other unknown individual specific factors may have a role in development of a *T. foetus* infection and carrier bull status.

In summary, the absence of macro and microscopic pathological changes and a limited immunological response to *T. foetus* infection in bulls result in a lack of clinical signs in infected bulls and development of inapparent, chronically infected bulls.

Herd-level impact

Clinical signs in a herd are the culmination of individual responses. Consequently, there are increases in: number of nonpregnant cows, incidence of pyometra and abortion, and delayed expected calving dates. Percent nonpregnant cows increased substantially (57%[31] and 45.3%[32]) with subsequent decreases in annual calf crop. A computer model predicted a 20 - 40% prevalence of *T. foetus* infection in bulls would lead to a 14 - 50% reduction in annual calf crop.[4] Additionally, a second common herd-based clinical sign is a prolonged calving interval. Cows exposed to *T. foetus* infected bulls had calving intervals of 96.5 and 98.9 days longer than nonexposed cows during the first and second years of herd infection, respectively.[21] Herd records would specifically identify this herd-based clinical sign, or it may be recognized due to an increased number of cows calving late in the calving season or lower weaning weights due to younger calves at weaning.

Diagnosis

One of the main aspects of controlling bovine trichomoniasis is identifying infected bulls. The following discussion on the diagnosis of bovine trichomoniasis will focus exclusively on the diagnostic process in a bull.

Sample collection

A modified version of a glass vaginal pipette and rubber bulb technique used in earlier studies to collect samples from female cattle was equally effective for aspirating samples from bull preputial cavities.[33] This device has been adapted to utilize a plastic infusion pipette, typically 45 - 53 cm in length and 0.497 - 0.571 cm in diameter, with a 12 or 20 ml disposable syringe. General collection technique includes the following: long hairs at the preputial orifice are clipped short to reduce contamination of the sampling device. A syringe is attached to an infusion pipette and the free end of the pipette is passed into the prepuce. When the free end of the pipette reaches the fornix of the prepuce, suction is applied with the syringe. While maintaining suction with the syringe, the pipette is moved forward and backward over the surface of the penis and prepuce several times to scrape smegma into the pipette. Prior to removing the pipette from prepuce, the negative pressure in the syringe is gently released to avoid pulling sample into the syringe. After this is accomplished, the pipette is removed, and sample examined.

A faint pink color in the sample is acceptable as the mucous membrane of the prepuce is mildly abraded during the collection process. However, overly aggressive scraping can lead to bleeding which may reduce the quality of the sample. If the sample is of adequate quality and volume, it is transferred to the appropriate

transport container. If a larger sample is needed, the pipette is reintroduced into the prepuce following the guidelines listed in the previous steps to collect additional smegma. After an adequate sample is recovered, it should be immediately placed in an appropriate medium for transport and testing.

Maintenance of the sample

Maintenance of *T. foetus* preputial specimens has been an important preanalytical concern for trichomoniasis diagnostic testing since the early days of investigation. Specimens not subjected to direct examination were frequently collected and maintained in a solution whose purpose was to preserve

T. foetus viability until they could be inoculated into laboratory media for incubation or placed directly into the laboratory incubator. However, the use of various solutions, broths and media strictly for specimen transport has been eliminated in the US following availability of direct inoculation into a growth medium at the collection location. This commercial transport and enrichment medium has a plastic cultivation envelope containing a proprietary medium (InPouch™TF, Biomed Diagnostics, Inc., White City, OR). Inoculated samples should be protected from extreme temperatures, as cold or heat may influence ability to detect *T. foetus*.[34,35]

Culture

The InPouch™TF is examined in a wet mount fashion,[36] by fixing the lower portion of the pouch in a plastic clip (size of a microscope slide and provided by the manufacturer) and placing the clip on a compound microscope. Systematic scanning of the pouch for motile organisms morphologically consistent with T. foetus should be done daily for several minutes and repeated for 6 consecutive days before declaring the specimen negative.[37]

Using bright field microscopy (100 - 400 x) to examine samples collected with InPouch™TF from virgin bulls, trichomonads were observed with morphological and motility characteristics consistent with *T. foetus*. Further testing of the trichomonads through staining, scanning electron microscopy, and PCR revealed that the organisms had 4 anterior flagellae, similar to lower-bowel commensal trichomonads (*Tetratrichomonas pavlovi* or *Tetratrichomonas buttreyi*). Perhaps these organisms were transferred to the prepuce in feces during sodomy.[38] Therefore, culture specificity is no longer regarded as 100%.

Polymerase chain reaction

To overcome concerns with *T. foetus* culture sensitivity and specificity, investigators examined the value of PCR as a trichomoniasis diagnostic assay, based on the assumption that amplification of DNA segments specific to *T. foetus* would reduce or eliminate false positives. Furthermore, testing specificity is increased by reducing false negatives, as the test is not influenced by number of organisms.[39]

Control

The specific steps to a trichomoniasis control program should be guided by the *T. foetus* infection status of the herd, the local prevalence of trichomoniasis, the herd owner's aversion to the risk of a disease incursion and balancing the cost and benefits of the various aspects of a trichomoniasis control program.

Infected herds

Based on the information provided above, recommendations for *T. foetus* elimination from infected herds include the following. Sample and test all herd bulls 3 times, regardless of the test used, and cull all test positive bulls or all herd bulls in positive herd. This limits the time and money spent on testing while eliminates any risk of misidentifying a bull as *T. foetus*-negative that would allow the organism to remain in the herd. However, this option carries a financial burden that may be unacceptable to the herd owner.

Cull all nonproductive females (i.e. those nonpregnant at the end of the breeding season or those that fail to deliver a live calf prior to the next breeding season) or establish 2 distinct female management groups based on their potential for *T. foetus* infection, with virgin heifers and cows delivering live calves in 1 group and nonproductive cows in the other. This option involves the risk of maintaining *T. foetus* in the herd through nonproductive cows and requires fastidious management, including absolute isolation of nonproductive cows from all other cattle and utilization of artificial insemination or bulls exclusive to this group.

Consider vaccinating all females with an approved trichomoniasis vaccine (TrichGuard®, Boehringer Ingelheim Vetmedica, Inc., Ingelheim, Germany). This will not prevent infection; however, it may reduce fetal losses associated with infection and the period of infection.[40,41] Finally, implement prevention strategies for high-risk herds (refer below).

High-risk uninfected herds

Herds are considered high risk for the introduction of *T. foetus* based on a relatively high local prevalence of the disease and utilization of management practices that increase the risk of introduction. Suggested strategies for prevention of *T. foetus* in high-risk herds include the following.

Plan a pasture utilization program to minimize contact with neighboring cattle. Utilize proper artificial insemination protocols with semen from a reputable source in specific management groups or eliminate the entire herd to greatly reduce the risk of *T. foetus* transmission. Maintain a young bull battery to reduce the rate of transmission and potential development of chronic carrier bulls.

Isolate and/or test cattle if unplanned commingling with

neighboring herds has occurred. Females should be isolated from the rest of the herd until after the breeding season and their pregnancy status can be confirmed. Bulls should be isolated and tested (3 tests at weekly intervals) to ensure that they are *T. foetus* negative.

Restrict the length of the breeding season to < 120 days to reduce the opportunity for transmission of the disease within the herd and to more easily monitor reproductive performance.

Institute a surveillance testing program. Surveillance testing should occur between the end of a breeding season and the beginning of the next and include a single test of all bulls in the herd. The advantage of surveillance testing performed closely after the breeding season is early detection of infection that allows time to develop and implement a complete trichomoniasis control program before the next breeding season. Bulls tested under this program must not be exposed to cows prior to the next breeding season. Alternatively, test all bulls immediately before the breeding season. This the advantage of timing the test to coincide with an annual breeding soundness examination, thereby reducing the number of times bulls are handled. However, this may not allow sufficient time for appropriate management of the disease before the start of the breeding season. Finally, implement prevention strategies for low-risk herds, as described below.

Low-risk uninfected herds

Herds are considered low risk for the introduction of *T. foetus* based on a low local prevalence of the disease and the utilization of management practices that reduce the risk of the introduction of trichomoniasis into the herd. Suggested strategies for prevention of *T. foetus* in low-risk herds include the following.

Develop communication networks with neighbors to ensure rapid notification if trichomoniasis is diagnosed in a neighboring herd. Monitor fences and cattle to rapidly identify when unplanned commingling with another herd has occurred so immediate steps can be taken to address the problem and reduce the risk of introducing trichomoniasis. Maintain herd records to monitor herd reproductive performance and identify animals within management groups for early detection of a potential trichomoniasis incursion and efficient management of the outbreak. Observe inter- and intra-state animal health regulations regarding trichomoniasis and other diseases. Regulations to protect the livestock industries of each state should be considered as a barrier to trichomoniasis introduction and not as a complete prevention program at the herd level.

Purchase replacement animals, preferably virgin bulls and heifers, from a reputable source. Purchase of nonvirgin bulls and cows, especially, from herds with unknown reproductive performance, increases the risk of trichomoniasis introduction. Accepted risks in purchasing nonvirgin replacements may be greatly reduced by having a source that has excellent herd reproductive performance with bulls tested negative or by purchasing pregnant cows.

Conclusion

Bovine trichomoniasis has plagued the US cattle population since the 1930's and continues to inflict reproductive loss and subsequently financial loss in the industry. Understanding the characteristics of this disease associated with its etiology, pathogenesis, diagnosis provides a logical and practical approach to control of the causative organism, *Tritrichomonas foetus.*

Conflict of interest

None to report.

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