Bovine trichomoniasis

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Abstract

The association of *Tritrichomonas foetus* with bovine reproductive failure was first identified ~ 130 years ago, and it became apparent in the US cattle population ~ 100 years ago. Research has increased our understanding of this disease. Current knowledge regarding clinical signs, diagnosis, and prevention is reviewed.

Keywords: *Tritrichomonas foetus*, bovine, diagnosis, treatment, vaccine

Introduction

Much progress has been made in our understanding of *Tritrichomonas foetus* (*T. foetus)* and trichomoniasis since the organism was implicated in causing reproductive failure in cattle. This review will cover the following topics: preputial distribution, bull susceptibility, sampling devices, culture, polymerase chain reaction (PCR) assay, immune response and vaccines, and treatment.

Preputial distribution

Distribution of *T. foetus* in the preputial cavity of bulls was documented in 1943.[1] Highest number of organisms were isolated in the midshaft and caudal portion of free penis, preputial area surrounding penis, galea glandis, and near preputial opening, in that order.[1] Limited effort was made to reexamine these findings until 1999, when the presence of *T. foetus* in the midshaft and caudal penis and to a lesser degree in the prepuce was evident in paraffin-embedded sections of preputial tissues.[2]

Bull susceptibility

It has long been recognized that bull age affected susceptibility to *T. foetus.* Older bulls had increased risk of becoming *T. foetus* carriers by contracting infection through breeding activity, their longevity in the herd, and their hierarchical dominance.[3] This age-related phenomenon of longevity and dominance in bulls was partially substantiated in 1970 reports from Australia.[4,5] As a control measure, bulls > 8 years of age were replaced by young bulls, negative for *T. foetus*. Prevalence of infection remained significantly lower in replacement bulls compared to older bulls (at their removal from the herd) suggesting that young bulls were less likely to be carriers.

Several studies assessed the correlation between bull's age and risk of *T. foetus* infection. Although these studies suggested that the risk for *T. foetus* carrier bull status increased in aged bulls, this conclusion was questionable due to limited bull numbers and potential for bias due to uneven distribution of age groups. Furthermore, these studies lacked controls. Many detailed studies[6-8] attempted to account for these factors. In 1 study, 2% of bulls < 3 years of age had *T. foetus* infection ($p < 0.025$) compared to 6.7% in bulls > 4 years.[6] Subsequently, [2] larger epidemiologic studies[7,8] had similar trends. Mean age of infected bulls was 5.5 ± 1.6 years ($p < 0.001$) and mean age of uninfected bulls was 3.9 ± 2.3 years.[7] Bulls > 5 years of age were 2.2 times (OR = 2.2, 95% CI; 1.1 - 4.3: $p = 0.022$) more likely to be *T. foetus* positive than bulls < 5 years of age.[8]

A proposed explanation for this relationship between age and *T. foetus* carrier status is the development of crypts in the epithelium of penis and prepuce in aged bulls. A similar relationship was established for bovine vibriosis.[9] Susceptibility increased substantially at > 5 years of age and was associated with and possibly linked to an increase in size and number of penile epithelial crypts.[9] Trichomoniasis investigators likewise implicated the development of crypts in aged bulls as a cause for age-related susceptibility to *T. foetus*[10,11] and immunohistochemical staining of paraffin embedded sections of preputial tissues apparently supported this explanation.[12] However, more recent work questioned the validity of this viewpoint.[13]

Sampling devices

Various sample collection techniques were proposed, including preputial swabbing using a cotton swab,[14] preputial scraping

with a specially designed instrument,[15] preputial lavage,[14] and preputial aspiration via a pipette.[16]

Swabbing consisted of passing a cotton swab into preputial cavity to the fornix area (via a speculum in the preputial orifice), moving the swab back and forth and rotating it around the glans penis, and then removing the swab.[14] Pipette aspiration was more reliable for direct examination of specimens than swabs if lower numbers of *T. foetus* were present in the prepuce.[17] Although the number of organisms present in the prepuce dictated pipette aspiration versus preputial swabs, growth of organisms in an enrichment medium (rather than direct examination) minimized this difference.[14]

A long, thin metal rod with shallow grooves (perpendicular to the shaft) at 1 end was used to scrape samples from the bovine preputial cavity.[15,18] Specimen collection with these devices was achieved by passing the grooved end of the rod into the preputial cavity, thrusting the head forward into the fornix area and drawing it back repeatedly (20 - 30 cycles) then withdrawing it from the prepuce and flushing the material from the instrument. This device was superior to preputial lavage to identify *T. foetus* in preputial specimens; however, supporting data and statistical analyses were not clear.[15] This device was also superior to pipette aspiration for direct examination of the specimen for *T. foetus* identification. Placing the specimen in a culture medium within 2 hours after collection resulted in 3 times more prolonged *T. foetus* survival in culture compared to specimens aspirated by pipette.[18] There were no significant differences between techniques (sensitivity for pipette aspiration was 91.6% [95% CI, 84.3 - 95.7%] and the scraping device was 93.3% (95% CI, 87.2 - 96.7%).[19]

Details regarding preputial lavage or douche technique vary among investigators. In general, a volume of sterile normal or phosphate buffered saline is instilled into preputial cavity with a syringe or rubber bulb via a pipette whose free end is positioned in the fornix area, the prepuce is massaged over the penis while holding the preputial orifice closed to prevent loss of saline, and lavage fluid is collected by aspirating with an instillation apparatus. Fluid retrieved by this method is typically placed in a test tube and centrifuged or preputial debris allowed to settle to form a pellet at the bottom of the container. The pellet is examined directly for *T. foetus* or inoculated into an enrichment medium and analysed.[14,20]

A comparison of the efficacy of preputial lavage and pipette aspiration for *T. foetus* recovery for direct examination concluded that lavage was more efficient for recovery of *T. foetus* at lower preputial concentrations.[14] Pipette aspiration had 65 positive specimens out of 83 (78.3% sensitivity [95% CI, 67.6 - 86.3%]), whereas lavage had 69 positive specimens out of 84 (82.1% sensitivity [95% CI, 71.9 - 89.3%]).[20] Authors concluded that these 2 collection methods were comparable as sensitivities were not significantly different. Preputial lavage and pipette aspiration specimens for culture and PCR analysis were compared. [21] Sensitivities for these 2 methods for PCR were not significantly different, although the difference approached significance when DNA extraction from the specimen was delayed for 5 days.[21]

A modified glass vaginal pipette and a rubber bulb (used by earlier investigators to collect *T. foetus* samples from female cattle) were used for aspirating samples from bulls' preputial cavities.[16] The free end of the pipette is passed into the preputial cavity to the fornix, aspirated with the rubber bulb as the free end of the pipette is moved back and forth over the surface of the penis and prepuce multiple times, and then the pipette is removed from the preputial cavity after gently releasing the suction from the rubber bulb. This device, adapted to use a plastic infusion pipette and a disposable syringe, has become the primary sampling technique in the US, due to its ease of use and lack of a better alternative.

Culture and PCR

Prior to PCR culture, microscopic examination was the diagnostic test of choice for bovine trichomoniasis and sensitivity estimates ranged from 67.7 to 98.4%.[22,23] Although multiple media and solutions were used for the transport and enrichment of samples, a proprietary medium in a specially designed in vitro cultivation envelope (InPouch™TF, Biomed Diagnostics, Inc., White City, OR) improved the ease of sample handling and is widely used throughout the US. Examination of the inoculated InPouch™TF (TF) is done by fixing the lower portion of the pouch in a plastic clip (size similar to a microscope slide). The clip is systematically scanned for several minutes for motile organisms (morphologically consistent with *T. foetus*), daily for 6 days, as recommended by the manufacturer.[22,24]

Sensitivity of TF cultures was 98.4% (150 bulls each sampled once). Cultures were examined once daily for 5 days maintained at 37˚C.[23] Culture sensitivities were 91.6% (95 CI, 84.3 - 95.7%)[19] and 95.8% (95% CI, 89.6 - 98.5%).[24] Furthermore, specimen handling must be impeccable.

Influence of various preanalytical factors on *T. foetus* detection in inoculated TF with equal numbers of preputial lavage and pipette aspirated specimens from bulls known to be naturally infected was determined. Sensitivities were not significantly different for preputial lavage versus pipette aspiration; therefore, data were combined to determine sensitivity. Total number of positive specimens by both collection methods in TF (n = 73) was divided by the total number of specimens collected from known positive bulls (n = 83) and the overall sensitivity was 88%.[20]

A large beef cattle herd, investigated for infertility, had a sensitivity of 73%.[7] Authors concluded that low culture sensitivities was likely due to reduced specimen quality due to various preanalytical conditions, including: extensive and remote cattle working facilities that made handling and transport of large numbers of specimens difficult; fractious bulls; harsh

environmental conditions; inconsistent bull identification; contamination of specimens with dirt and feces; and a large number (n = 750) of specimens.[7]

Similarly, the overall sensitivity of culture was 70.4% in a herd investigated for infertility. However, sensitivity for specimens collected during weeks 1 and 3 was 83.3% and for week 2 was 44.4%. Investigators concluded preanalytical factors (e.g. variations in collection, handling, culture techniques) and fluctuating preputial *T. foetus* populations might have decreased week-2 sensitivity. This study highlighted the significance of preanalytical factors over analytical factors when reasonable analytical techniques are used.[25]

Lowest reported sensitivity (67.8%, 95% CI; 51.1 - 84.1) for TF culture was from experimentally infected bulls sampled weekly for 6 weeks via pipette aspiration, with the TF incubated at 3˚C for 7 days and examined on days 1, 3, 5, and 7 postinoculation. Sensitivity was calculated as the number of positive culture samples out of the total number of samples from experimentally infected bulls. This was probably an underestimation of sensitivity, as 4 bulls produced no positive specimens by culture over the 6-week study and were most likely not *T. foetus* infected.[22]

Although the sensitivity of *T. foetus* culture has a wide range due to preanalytical and analytical factors that varied among studies, until recently, diagnostic specificity of *T. foetus* culture had been assumed to be nearly 100%. Based on brightfield microscopy (100 - 400 x), other trichomonads in specimens from virgin bulls had morphological and motility characteristics similar to *T. foetus*. However, further testing of these trichomonads, including staining, scanning electron microscopy, and PCR revealed 4 anterior flagellae. It was speculated that these organisms were commensal (intestinal) trichomonads (*Tetratrichomonas pavlovi* or *Tetratrichomonas buttreyi*), likely present in feces and transferred to the prepuce during sodomy.[26]

To overcome *T. foetus* culture sensitivity and specificity concerns, investigators examined the value of PCR as a diagnostic assay. The assumptions were: amplification of DNA segments specific to *T. foetus* would reduce or eliminate false positives, thereby increasing specificity and identification of positive specimens without the presence of a living organism; or when specimens contained fewer organisms, that would increase testing sensitivity by decreasing false negatives.

A PCR assay was developed utilizing primers TF1 and TF2 to amplify a 162-base pair (bp) product from an unspecified region of *T. foetus* DNA. Assay sensitivity (88.6%) was comparable to traditional culture, despite its ability to detect as few as 1 organism in pure medium and 10 in medium with smegma. No false positives were detected from 8 *T. foetus* negative bulls.[27]

Primers TFR3 and TFR4 were developed to target the 5.8S rRNA, ITS1, and ITS2 regions for PCR amplification while incorporating an uracil DNA glycosylase system to prevent DNA carryover from previous reactions and a DNA enzyme immunoassay (DEIA) for amplicon detection. The 347 bp amplification product was obtained from 8 isolates of *T. foetus*, *Tritrichomonas suis*, and *Tritrichomonas mobilensis*, but no amplification product was produced from PCR assays of specimens containing other trichomonads, bacterial DNA, or bovine DNA. The assay was able to detect quantities of DNA equivalent to a single *T. foetus* organism in pure media and as few as 50 organisms per ml in specimens containing smegma, bacteria, and other debris. False positive specimens that had been an issue with the TF1-2 PCR were not observed with the TFR3-4 assay, indicating higher test specificity.[28]

A PCR (TFR3-4 PCR) with a reported detection limit of 2 organisms per ml of specimen had a sensitivity of 31 - 90% and a specificity of 98%.[21] For a detection limit of 5 organisms per ml, the diagnostic sensitivity was 98.3% and the diagnostic specificity was 93.7%.[29]

Real-time PCR (rtPCR) was used for *T. foetus* diagnosis using primers TFF2 and TFR2 and a fluorescent probe after employing a heat lysis method for crude cell lysate preparation. The sequence of this TFR2 differed from the previously mentioned TFR2. The detection limit for rtPCR was a single cell equivalent for laboratory-spiked preputial smegma specimens with less than a cell equivalent per assay reliably detected from several heat-lysed specimens; this was a 2500-fold higher analytical sensitivity than culture and similar to TFR3-4 PCR in analytical sensitivity. A field-based comparison[30] suggested that rtPCR was superior to culture. Specimens from 159 animals in known *T. foetus* infected herds had 3 positive specimens for culture and 14 positive specimens for rtPCR. However, no repeat testing of test positive animals was undertaken to confirm their TF status, leaving open the possibility of rtPCR false positive results. No rtPCR diagnostic sensitivity or specificity estimates were provided.[30]

Immune response and vaccines

Precise immunological mechanism for *T. foetus* clearance from the female bovine reproductive tract is unknown. Antibodies and complement activated by *T. foetus* surface antigens conferred protection from the organism.[31] Elimination occurred when trichomonads were opsonized with antibodies and complement before exposure to neutrophils.[32]

Repeated *T. foetus* infections appeared to cause an anamnestic response. The interval cows remained infected decreased in subsequent exposures, when mean durations of infection for first, second, and third exposures were 20.3, 9.8, and 11 weeks respectively.[33] After a previous infection with *T. foetus*, trichomonads were cleared from heifers' reproductive tracts within 3 weeks after reinfection.[34] Other researchers reported that 6 cows were resistant to reinfection when exposed to *T. foetus* 4 months after recovery from a previous infection.[35] However, this immunologic memory appeared to be short lived. [36] Estimated

length of partially protective immunity was < 15 months, based on cow fertility following *T. foetus* exposure.[36]

Although clearance of the organism from the female reproductive tract is typical, infections persisted for up to 300 days[37] or up to 22 months postbreeding.[38] Carrier cows remained infected through a normal pregnancy with *T. foetus* isolated up to 9 weeks[39] or 63 - 97 days[40] after delivering an apparently normal calf.

Despite not knowing the exact immune mechanism, a vaccine was reported to reduce losses associated with *T. foetus* infection in heifers by lowering the rate of *T. foetus* infection, decreasing the estimated duration of infection, and interfering with the ability of *T. foetus* to cause early embryonic death.[41] A commercial vaccine (TrichGuard®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) substantially increased pregnancy and calving rates in heifers and reduced losses associated with *T. foetus* infection.[42] Vaccination led to resistance to infection up to 5 years of age[43] and resistance to *T. foetus* colonization.[44] However, for bulls, a critical review and meta-analysis of the whole-cell killed vaccine reported a likely overestimation of the benefits of vaccination and a low to very low quality of evidence.[45]

Treatment

Although various substances can clear *T. foetus* from infected animals, nitroimidazole derivates are commonly used.[46] Single ipronidazole treatment was effective in 92.8% bulls and 3 daily treatments in 100% of bulls.[47] Regardless of their effectiveness, toxicity and resistance concerns remain unknown.[46] Furthermore, the use of nitroimidazole derivates in food producing animals is currently not approved in the US. Use of a commercially available vaccine as a therapeutic agent did not significantly decrease infection prevalence.[48] Currently, no effective legal treatment for bovine trichomoniasis is available in the US.

Conclusion

Ever since *Tritrichomonas foetus* was first described as a cause for reproductive failure in cattle, a substantial effort has been made to better understand the organism's interaction with the bovine reproductive tract and to improve diagnosis, prevention, and treatment. Despite great strides, there are many opportunities for further research in pathogenesis, immunology, diagnostics, prevention, and treatment.

Conflict of interest

None to report.

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