Student Clinical Paper

A Preliminary Survey of North Carolina Slaughterhouse Bulls for *Tritrichomonas foetus*

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Summary

A total of 450 bulls presented to a North Carolina slaughterhouse were tested for *Tritrichomonas foetus,* the causative agent of bovine trichomoniasis. A sample of preputial smegma was collected from each bull, and inoculated into a proprietary medium, specifically designed for the diagnosis of *T. foetus* infections. Each sample was monitored periodically throughout the 5 day incubation interval for growth of the organism. No cases of trichomoniasis were identified at any stage of the screening process. The ability of the organism to survive and multiply in the culturing system, both as a pure culture and in the presence of smegma from an uninfected bull, was verified using a positive control strain *T. foetus* 1119.

Introduction

Tritrichomonas foetus is a flagellated, obligately anaerobic protozoan that is the causative agent of bovine trichomoniasis.[1] Trichomoniasis is an important venereal disease of cattle, with an estimated economic impact on the beef industry of $2000 to $2500 per infected bull.[9] The organism is carried asymptomatically by older bulls, and is thought to reside in the penile and preputial epithelial crypts. Because these crypts are much larger and deeper in older bulls, only bulls over 4 years of age are considered to be significant carriers of the disease. Transmission of the organism is primarily through breeding a cow to an infected bull, although it is also thought that the organism can be spread mechanically by young bulls, insemination pipettes, or other fomites.[1]

The primary manifestation of trichomoniasis in the cow is vaginitis, cervicitis, and pyometra, leading to early embryonic death or abortion from 15-80 days post-conception. The cow will typically remain infertile for 1 to 3 heat cycles due to advanced infection and inflammation, but will normally clear the infection. A short lived immunity of 6-12 months develops, after which the cow may be reinfected at the next breeding to an infected bull.[1] The cost of treating, culling, and replacing infected cattle may have a large economic impact on the producer. However, the biggest source of economic loss is the loss or reduction in the calf crop.[7]

The prevalence of trichomoniasis in the United States is difficult to estimate, since only the state of Idaho has instituted mandatory reporting of trichomoniasis cases. Limited sampling of bulls in sale barns and slaughterhouses has indicated a prevalence of 5-8%, but this figure apparently varies by region and management style.[2,4,8,10] Operations that use large, commingled herds with natural breeding, such as those in the Western rangeland, may have infection rates of up to 44%.[8] Thus, trichomoniasis has the potential to be a very large factor in the profitability of a beef herd.

The objective of this study was to determine the prevalence of trichomoniasis in bulls presented to a North Carolina slaughterhouse. Trichomoniasis has not been documented in North Carolina, although the movement of cattle from areas where the disease is endemic certainly provides a potential route for the disease to become established in the area. Many North Carolina cow/calf producers utilize a year-round calving schedule, therefore the disease may be present but simply not recognized by the producers. Considering the potential economic impact of trichomoniasis on beef cow/calf operations, knowledge of the prevalence of the disease would be useful to producers in this state for planning vaccination and control strategies.

This research was started by David Hobbs and John Stinson during their senior year. After their graduation, it was continued by Elisa Fox, Class of 1997, under the direction of Dr. Glenn Rogers.
Awarded the $200 first prize by the AABP for student clinical paper.

Materials and Methods

Collection of Samples

Martin's Abattoir in Godwin, North Carolina, was the site chosen for this study. A total of 12 trips were made to the facility, and 450 bulls were sampled. As the bull carcasses passed through the facility, the penis and prepuce were removed and placed in individual plastic obstetric sleeves. The bull identification number and state of origin were recorded. Then, the preputial smegma was collected from each sample using a dry, sterile infusion pipette, and a sterile 35 cc syringe to induce negative pressure. The epithelial surface of the prepuce and penis was vigorously scraped down to the level of the fornix, and the collected material was inoculated into a proprietary *T. foetus* isolation medium (InPouch, BioMed Diagnostics, Santa Clara, California).

Screening of Samples

The samples were screened immediately at 100 and 400X magnification, and the quality of the sample was judged. A sample was considered to be adequate if at least one milliliter of inoculum was collected, and if microscopic analysis revealed sheets of epithelial cells in the inoculum. After inoculation, the pouches were incubated at 35°C in a 5% CO_2 incubator. The cultures were re-examined at 2 days and 5 days post-inoculation for any motile organisms. For comparison, a positive control strain, *T. foetus* 1119, was obtained from Dr. Bruce Abbitt of the Texas Veterinary Medical Diagnostic Laboratory. The organism was serially passaged in Diamond's medium, and was periodically inoculated into the InPouch medium system. Also, in order to determine the ability of the organism to grow in the medium in the presence of smegma, the positive control strain was inoculated into a medium pouch containing a smegma sample from an uninfected bull.

Results

A total of 450 bulls representing 7 southeastern states were examined in this study (see Table 1). A smegma sample was collected from each bull using a dry infusion pipette and a syringe, and the collected material was cultured for *T. foetus*. Culturing the sample to allow growth of the organism is considered to be the most reliable method of detecting a *T. foetus* infection, with a sensitivity of up to 97%.[7] The InPouch culturing system, specifically designed for the isolation of *T. foetus,* has been demonstrated to have a sensitivity identical to the traditional method of culturing the organism in glass tubes of Diamond's medium.[3,5] The ability of this culturing system medium to support growth of *T. foetus* was verified by inoculating a positive control strain of the organism. This test inoculum remained viable throughout the experimental period. In addition, our positive control strain demonstrated growth in the presence of smegma from an uninfected bull.

Each sample was examined a total of three times: immediately after inoculation to judge the quality of the sample, and after 2 and 5 days of growth. *Tritrichomonas foetus* was identified in any of the samples at any stage of the screening process.

Table 1. Breakdown of bulls sampled by state of origin.

State	Number of Bulls Sampled
North Carolina	130
Virginia	90
South Carolina	73
Kentucky	49
Georgia	48
Tennessee	31
Florida	20
Pennsylvania	9
Total	**450**

Discussion

We were intrigued by the lack of identified cases of *T. foetus* in our samples. The techniques used in this study were similar to those used in previous studies,[2,10] and the work was carefully supervised by a veterinarian with experience in culturing bulls for *T. foetus*. Our positive control verified the ability of *T. foetus* to grow in the proprietary culturing system, both as a pure culture and when co-inoculated with smegma from an uninfected bull. Thus, we feel that the lack of identified cases of *T. foetus* is not due to experimental error, but instead due to a lack of infected bulls presented for slaughter during our sampling time frame.

Out of the 450 bulls cultured for *T. foetus,* 130 were from North Carolina. There are approximately 500,000 beef cows in North Carolina, and assuming a 1:20 ratio of bulls to cows, there should be approximately 25,000 beef bulls in this state. We assumed a 3% incidence of trichomoniasis would have been present in North Carolina, which would have been lower than the previously reported 5-8% prevalence in beef bulls. Using a sample size calculation for a population survey based on random sampling, we found that these results are significant ($p<.01$). Thus, our North Carolina sample size was appropriate for this experiment.

One area that we could not control was the composition of our sample pool. Since the samples were collected from bulls at a slaughterhouse, it was not possible to determine the age and breeding history of the bulls. Only bulls greater than 4 years of age are consid-

ered to be persistently infected, thus the presence of a large number of young bulls could influence our results. We reasoned that bulls sent to slaughter were more likely to be older, and casual visual inspection of the bulls prior to slaughter seemed to confirm our observations. However, we can not rule out the possibility of age bias in the sample pool.

From this preliminary study, it is not possible to rule out the presence of a low-level of endemic trichomoniasis in North Carolina. Future study and surveillance would be advantageous in determining the extent of any possible infection. The chances of finding infected bulls would be maximized by testing primarily bulls older than 4 years of age in herds having reproductive problems suggestive of trichomoniasis, including prolonged calving intervals and uneven calf crops.

References

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Abstract

The effect of ivermectin treatment of late pregnant dairy cows in south-west Victoria on subsequent milk production and reproductive performance

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A total of 498 dairy cows in 5 predominantly pasture-fed herds were allocated to pairs. One cow in each pair was treated with a single dose of ivermectin during the dry period. Treated and untreated cows were managed as a single group throughout the trial. Most cows calved between 45 and 115 days after treatment. When data from all herds were pooled, treated cows produced an extra 74 L of milk over the first 100 days of the subsequent lactation (95% confidence interval 20 to 128). Means were greater among treated groups relative to untreated groups in all 5 herds. However, when analyzed individually, differences were statistically significant ($P < 0.05$) in 1 herd only. Over the complete lactation, mean milk volume for treated cows was 86 L greater than for untreated cows (95% confidence interval of difference -57 to 229; $P \pm 0.24$). Untreated cows produced 2473 L and 5883 L for the first 100 days of lactation and for the complete lactation, respectively. Milk production responses to treatment did not vary significantly with parity, body condition score, previous production index, calving date category or with plasma pepsinogen concentration or faecal egg count at the time of treatment. Faecal egg counts and plasma pepsinogen concentrations were low at the start of the study. The interval from calving to conception was 4.8 days less in treated cows (95% confidence interval 1.2 to 8.2) relative to untreated cows when data from all 5 herds were pooled. Differences within individual herds were not statistically significant.