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Prevalence of *Tritrichomonas foetus* in several subpopulations of Alabama beef bulls

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Abstract

The objective of this study was to estimate the prevalence of *Tritrichomonas foetus* infections in Alabama (USA) beef bulls through prospective and retrospective surveys. The prospective survey included 240 Alabama beef bulls that were sampled between January 2005 and March 2006. Preputial smegma was collected from the 240 bulls with a dry pipette and cultured in an InPouch™ TF *T. foetus* culture pouch (BioMed Diagnostics; White City, OR, USA). The samples were evaluated microscopically once a day for 6 days for growth resembling *T. foetus*. To avoid false-positives due to fecal trichomonads, all suspect cultures were sent to both the Alabama Department of Agriculture Veterinary Diagnostic Laboratory in Auburn, AL, USA and the Auburn University College of Veterinary Medicine Parasitology Laboratory (Auburn, AL, USA) for polymerase chain reaction (PCR) confirmatory assays. Of the 240 bulls cultured in the prospective survey, 3 (1.25%) cultures were considered suspect on microscopic evaluation. However, PCR-based assays were negative for *T. foetus*, suggesting that the samples most likely contained fecal trichomonads. The retrospective analysis included 374 *T. foetus* cultures performed at the Alabama Department of Agriculture Veterinary Diagnostic Laboratory between October 2002 and March 2005. Of the 374 bulls included in the retrospective analysis, only 1 (0.27%) was confirmed positive by a PCR-based assay.

Keywords: Trichomoniasis; Trichomonosis; Venereal disease; *Tritrichomonas foetus*; *T. foetus*

1. Introduction

Bovine trichomoniasis, also known as trichomonosis, is a venereal disease caused by the protozoan *Tritrichomonas foetus*. Trichomoniasis is a major cause of fetal wastage throughout the world; it causes substantial economic losses wherever natural breeding conditions exist. Infected bulls are often asymptomatic carriers of *T. foetus*, however, they are very efficient in transmitting the organism to a cow or heifer during coitus and it is the female that suffers the consequences of infection. Infections in cows and heifers can result in early embryonic death, abortion, pyometra, fetal maceration, or infertility [1–4], influencing the profitability of a cattle operation. *T. foetus* can persist in endemic herds without detection for many years and have a substantial economic impact on a cattle operation due to three factors: (1) reduced calf crop due to early embryonic loss or abortion; (2) reduced weaning weight

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due to delayed conception; (3) culling and replacement of infected cattle.

Since no legal treatment exists, preventive and control measures focus on testing and culling positive animals, administration of a killed vaccine, and education of cattle producers and veterinarians regarding risk assessment and herd biosecurity. Therefore, a major component of education is establishing the prevalence of trichomoniasis in a region to aid in risk assessment.

Prior to this investigation, there were no reported prevalence rates of trichomoniasis in Alabama (USA) beef cattle. Therefore, based on epidemiological studies conducted in other states [6–11] that reported individual bull prevalence rates of 0–7.8%, it was hypothesized that the prevalence of trichomoniasis in Alabama beef bulls was approximately 3%. However, even at a prevalence of 3% or less, trichomoniasis could still cost the Alabama beef industry millions of dollars annually. Therefore, retrospective and prospective surveys were undertaken to estimate the prevalence of *T. foetus* in several non-randomized subpopulations of Alabama beef bulls.

2. Materials and methods

2.1. Sample size determination

According to the Alabama Agricultural Statistics Bulletin, there were approximately 724,000 beef cows in Alabama in 2005 [12]. Based on that figure, and assuming a bull to cow ratio of 1:20, there were approximately 36,200 herd bulls in Alabama in 2005. The sample size was then calculated using the Epi Info™ Version 3.3 software from the Centers for Disease Control and Prevention (Atlanta, GA, USA). Given an estimated prevalence of 3% (and not less than 0.1%), a confidence level of 95%, and a population of 36,200 herd bulls, 132 bulls were needed to estimate the prevalence of *T. foetus* in Alabama beef bulls.

2.2. Prospective survey animals

Bulls were sampled from January 2005 to March 2006. A bull's individual identification, age, breed, location, and herd size were recorded at the time of collection. All owners volunteered to have their bulls tested for *T. foetus*. Of the 240 bulls sampled, 148 presented to the Auburn University College of Veterinary Medicine Large Animal Clinic (Auburn, AL, USA) for routine breeding soundness evaluations, 76 presented to private Alabama veterinarians for routine breeding soundness evaluations, and 16 presented to a private Alabama veterinarian due to an undefined herd reproductive problem.

2.3. Sampling technique

Bulls were properly restrained in a livestock chute to avoid injury to the bulls or personnel and then sampled as described by Peter [13]. The external preputial area was cleaned with disposable paper towels without soap or disinfectants. A new pair of examination gloves was used for each bull, and a sterile, dry, plastic 21-in. infusion pipette with a 12 or 20 mL syringe attached to one end was placed into the preputial fornix. The pipette was scraped vigorously across the preputial epithelium without aspiration, and then negative pressure was applied with the syringe to collect preputial smegma. The negative pressure was released before removing the pipette from the sheath, to avoid unnecessary aspiration of urine or other contaminants. After removing the pipette from the sheath, the sample was placed immediately into the transport/culture medium as described below. A new syringe and pipette were used for each bull.

2.4. Culture media

The selected transport and culture medium for this research was the self-contained InPouch™ TF *T. foetus* culture pouch (BioMed Diagnostics; White City, OR, USA). The InPouch™ TF medium contains trypticase, proteose peptone, yeast extract, maltose, and other sugars, amino acids, salts, antifungal, and antimicrobial agents in a normal saline phosphate buffer. The medium is selective for the transport and growth of *T. foetus* while inhibiting the growth of contaminating microorganisms. The pouch is constructed with a clear plastic film with water vapor and oxygen-barrier qualities that help maintain the proper microaerophilic environment. The pouch contains two V-shaped chambers that are separated by a channel that allows liquid to pass through if pressure is applied. An inoculum of 100 or fewer microorganisms is sufficient to result in a positive test [14].

The pouch was inoculated as described by Borchardt et al. [14]. The InPouch™ TF medium was manually expressed to place approximately 1 mL (from a total of 4 mL) in the upper chamber. The pouch was then opened at the notch just above the closure tape, and the pipette tip inserted into the upper chamber. Approximately 0.5 mL (no more than 1.0 mL) of the preputial sample was inoculated into the upper chamber. If the preputial smegma adhered to the wall of the pipette, a