



## Impact of a killed *Tritrichomonas foetus* vaccine on clearance of the organism and subsequent fertility of heifers following experimental inoculation



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### ABSTRACT

*Tritrichomonas foetus* is a sexually transmitted reproductive pathogen of cattle that causes transient infertility, early embryonic death, metritis, pyometra, and sporadic abortions. The objective of this research was to assess the impact on reproductive health of vaccinating naïve heifers with a killed *T. foetus* vaccine (TrichGuard) before experimental exposure followed by breeding. A total of 40 beef heifers were randomly assigned into two treatment groups. Heifers were then vaccinated with two doses of TrichGuard or sham vaccinated with 0.9% sterile saline according to their respective groups. Sixty days following vaccination or sham vaccination, heifers were intravaginally inoculated with  $2 \times 10^6$  organisms of a cloned isolate of *T. foetus* of bovine origin (CDTF-4) during synchronized estrus. Three days following inoculation of *T. foetus*, bulls free of *T. foetus* were introduced for natural breeding. Three bulls were maintained with the 40 heifers (20 vaccinated; 20 sham vaccinated) for a 49-day breeding season. Cervical mucous samples were obtained from each heifer at Day 0 and at 29 additional time points throughout the study for *T. foetus* culture. Pregnancy assessments were performed routinely by using transrectal palpation and ultrasonography. Pregnancies were detected in 19/20 (95%) vaccinated heifers and 14/20 (70%) sham-vaccinated heifers ( $P = 0.046$ ). Only 4/20 (20%) of the sham-vaccinated heifers gave birth to a live calf compared with 10/20 (50%) of the vaccinated heifers ( $P = 0.048$ ). Thus, embryonic or fetal loss was detected in 9/19 (47%) vaccinated heifers and 10/14 (71%) sham-vaccinated heifers ( $P = 0.153$ ). The interval of time between inoculations with *T. foetus* and conceptions of pregnancies that were maintained until birth did not differ significantly between groups (vaccinated = 18.7 days; sham-vaccinated = 17.3 days;  $P = 0.716$ ). The infectious challenge in this study proved to be very rigorous as a positive culture was detected from all heifers. The culture-positive results on the last culture day did not differ significantly ( $P = 0.115$ ) between vaccinated heifers (63.9 days) and sham-vaccinated heifers (79.2 days). All uterine culture samples collected from the 26 nonpregnant heifers on Day 207 postinoculation did not result in the detection of *T. foetus*. These findings indicate that the killed, whole cell vaccine used in this study (TrichGuard) was effective in improving reproductive health evidenced by significantly reducing losses associated with *T. foetus* infections.

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## 1. Introduction

Bovine trichomoniasis is a sexually transmitted disease caused by the extracellular protozoa *Tritrichomonas foetus*. This protozoa is an obligate parasite of the reproductive tract of cattle. Infected bulls are asymptomatic carriers of *T. foetus*. This organism is isolated from the bull's penis and the folds on the mucosal surfaces of the prepuce. Infected bulls are capable of transmitting the organism to a cow during coitus [1]. Infections in cows cause endometritis, cervicitis, and vaginitis which may result in early embryonic death, abortion, pyometra, fetal maceration, and/or transient infertility [1]. The major economic losses associated with *T. foetus* are due to: 1) reduced calf crop due to early embryonic loss or abortion, 2) reduced weaning weight due to delayed conception, and 3) culling and replacement of infected cattle. Due to the inability to use efficacious drugs, such as nitroimidazoles, for control and prevention of *T. foetus* infections in food animals, most control efforts have been targeted at identification and elimination of positive bulls. In addition, implementation of management strategies to prevent introduction of the organism into a herd, along with systemic immunization of cows and bulls are often utilized to minimize the chance of an outbreak.

The lack of effective therapy against bovine trichomoniasis prompted the development of vaccines. An inactivated, whole cell vaccination of *T. foetus* has been shown to be effective in reducing the number of infected females, reproductive losses, and duration of genital infections in heifers when vaccinated systemically [2–4]. However, another study found no advantage in using a whole cell vaccine in cows [5]. Another study evaluated the use of a cellular membrane laboratory preparation of *T. foetus*. In this study, the cell membrane laboratory preparation appeared to be beneficial but no more efficacious than the whole cell vaccine [6]. The practical advantage of vaccination for *T. foetus* has been questioned due to the lack of demonstrated impact of experimental vaccines on improving reproduction in the face of exposure of heifers and cows to this protozoal pathogen [2–5]. The purpose of this work was to assess the reproductive impact of pre-breeding vaccination with a commercial killed, whole cell vaccine (TrichGuard, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA) administered 60 days before experimental exposure to *T. Foetus*.

## 2. Materials and methods

### 2.1. Animals

All study procedures were approved by the Auburn University Institutional Animal Care and Use Committee (Protocol Review #2011–1967). 40 virgin, 12- to 14-month-old crossbred, beef heifers and three mature Angus bulls greater than 3 years of age were acquired from Auburn University Animal Health Research for the study. Heifers were selected for inclusion in the study based on reproductive track scores [7]. Only heifers with reproductive tract scores greater than three out of five were utilized in this study. Three sexually mature bulls were selected for use in this study based on their classification as satisfactory

potential breeders based on physical soundness, scrotal circumference, sperm motility, and sperm morphology. As per standard operating procedures for Auburn University Animal Health Research, breeding bulls are not vaccinated against the common viral respiratory/reproductive pathogens. Bulls were vaccinated against clostridial diseases as calves and are boosted annually. Before purchase and use for breeding, all bulls were tested for BVDV persistent infection by antigen capture ELISA on ear notch samples and virus isolation from whole blood. Semen was tested for prolonged BVDV testicular infection by RT-nPCR. The heifers were unvaccinated against the common viral respiratory/reproductive pathogens, but were vaccinated against clostridial disease as calves and boosted. They were evaluated for BVDV by virus isolation from serum. Before use, the bulls were consistently culture negative for *T. foetus* as well as *Campylobacter fetus* and *Histophilus somni*. Calves (n = 14) subsequently born to the heifers on this study were maintained with their dams from birth until weaning and until after completion of this study.

### 2.2. Experimental design

Heifers were allocated into two groups. Group one consisted of those heifers vaccinated with TrichGuard vaccine (Boehringer Ingelheim, Ridgefield, CT, USA) (n = 20). Group two (control group) consisted of those heifers sham vaccinated with 0.9% PBS (n = 20). The study design was a generalized random block study design with weight used as the blocking factor.

The beginning of the study was designated as Day 0. On Days 0 and 14 of the study, heifers were vaccinated or sham vaccinated. Estrus was synchronized in the heifers. On the day of synchronized estrus, Day 74, all heifers were intravaginally inoculated during the synchronized estrus with  $2 \times 10^6$  organisms of a cloned isolate of *T. foetus* of bovine origin (CDTf-4). On Day 77, the 40 heifers (20 vaccinated; 20 sham vaccinated) were comingled and exposed to the three bulls for natural mating for 49 days. Estrous behavior was detected using radio frequency technology, Heat Watch (Cowhips, LLC, Manalapan, NJ, USA) to validate gestational ages and assess the timing of behavioral estrus after inoculation with *T. foetus*. Pregnant heifers were retained to allow pregnancies to be maintained to term. All nonpregnant heifers were euthanized on Day 207 after inoculation.

### 2.3. Animal housing and care

Heifers were comingled and group housed at the North Auburn Beef Unit in Auburn, Alabama. Bulls were introduced to the heifers during the breeding period (Day 77 through Day 126). Animals were maintained on pastures containing fescue and Bahia grass. Animals were also fed equivalent amounts of ryegrass haylage, soyhull pellets, corn gluten pellets on a per animal basis. On Days 14 and 38, all heifers were vaccinated with a multivalent MLV vaccine (Express FP 5-VL5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA) to minimize potential for other pathogens to affect pregnancy rates in the study. Throughout the study, the general health of all cattle were evaluated twice daily by animal caretakers.

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