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Serum, uterine, and vaginal mucosal IgG antibody responses against *Tritrichomonas foetus* after administration of a commercial killed whole *T foetus* vaccine in beef cows

R.A. Palomares [a,*], D.J. Hurley [a], L.T. Crum [b], E. Rollin [a], T. Collop [c], A. Williard [a], J. Felton [a], J. Parrish [a], L.B. Corbeil [b]

[a] *Department of Population Health, College of Veterinary Medicine, The University of Georgia, Athens, Georgia, USA*
[b] *Department of Pathology, University of California, San Diego, California, USA*
[c] *Veterinary practice, Rutledge, Missouri, USA*

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ABSTRACT

The objective of this study was to determine the level and duration of IgG antibodies induced against killed whole *Tritrichomonas foetus* and *T foetus*–purified surface antigen (TF1.17) in serum, vaginal, and uterine secretions after systemic immunization of beef cows with a vaccine containing killed whole *T foetus.* Twenty nonpregnant beef cows were randomly assigned to vaccine or control groups as follows: Vaccine (n = 10): cows received 2 mL of a commercial vaccine containing killed whole *T foetus* subcutaneously and a 2-mL booster 2 weeks later. Control (n = 10): cows received 2 mL of sterile saline on the same schedule. Vaginal secretions and blood samples were collected on Days 0, 8, 15, 22, 29, 36, 43, 50, 60, 75, 89, 110, 146, and 182 relative to day of primary vaccination. Uterine flush fluid was collected on Days 0, 15, 29, and 43 after the day of primary vaccination. Samples were assayed for IgG antibodies to the killed whole *T foetus* and surface antigen TF1.17 using enzyme-linked immunosorbent assay. Serum whole *T foetus*–specific IgG levels were significantly increased (between Days 15 and 182) following vaccination with *T foetus* or with saline. No differences between vaccinates and controls in uterine responses to whole-cell antigen were detected. Serum anti-TF1.17 IgG responses to vaccination were significantly higher than Day 0 throughout the immunization period ($P < 0.001$) and were higher than responses in control animals on each day post immunization through Day 146 ($P < 0.001$). A significant rise in TF1.17-specific IgG levels was observed in vaginal and uterine fluids from Day 15 post vaccination compared to the Day 0 levels. These levels remained significantly elevated in vaginal and uterine fluids through Days 75 ($P < 0.05$) and 43 ($P < 0.001$) after primary vaccination, respectively. Antibody levels in serum, vaginal, and uterine secretions against TF1.17 remained low in the control group throughout the study. In conclusion, vaccination of beef cows with a commercial vaccine containing *T foetus* induced significant increase in the levels of IgG to the *T foetus* TF1.17 surface antigen in serum, vaginal secretions, and uterine fluid, which remained elevated through Days 43, 75, and 182 in uterine fluids, vaginal secretions, and serum, respectively. Since purified TF1.17 antigen has been shown to protect against experimental *T foetus* infection in heifers, the vaccine-induced TF1.17-specific IgG response is likely to be important in the prevention of trichomoniasis in beef cattle.

* Corresponding author. Tel.: +1 706 542 6320; fax: +1 706 542 8907.
E-mail address: palomnr@uga.edu (R.A. Palomares).

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1. Introduction

Bovine trichomoniasis is a sexually transmitted disease caused by the extracellular protozoa *Tritrichomonas foetus*, which decreases cattle-reproductive performance resulting in substantial economic losses in livestock industry. The prevalence of *T foetus* infected beef herds has been reported between 16% and 44% of tested herds in California and Nevada [1,2]. Infections with *T foetus* result in vaginitis, cervicitis, and endometritis which are associated with reproductive failure characterized by embryonic death and abortion [3–5]. In beef herds, outbreaks of *T foetus* are responsible for low pregnancy rates, prolonged calving to conception intervals, and a significant proportion of cows becoming pregnant and calving at the end of the breeding and calving seasons [4]. Calves born late during the calving season are weaned at younger ages and with lower weights than expected. Therefore, bovine trichomoniasis has a direct negative impact on the profitability of beef farms. Field studies have shown that cows usually clear the *T foetus* infection within a few months; however, some cows may act as unapparent carriers [6–9]. Since *T foetus* is an extracellular microorganism, antibodies are important for protection. After infection with *T foetus,* cows respond with a systemic and mucosal immunity characterized by a significant IgG and IgA antibody response in vaginal and uterine secretions, which contributes to clearing the infection and reestablishing fertility after few weeks [10–15]. However, the immunity acquired during the infection with *T foetus* in cows does not consistently prevent the reproductive losses during that breeding season [16]. This immune response to infection has been reported to be of short duration (up to 6 months); therefore, cows might be susceptible to infection in subsequent years, which represents a limiting factor in the prevention and control of this disease.

The absence of effective, approved treatment for bovine trichomoniasis highlights the necessity for appropriate preventive and control measures that includes the establishment of adequate biosecurity measures, identification and elimination of *T foetus*–positive bulls, implementation of artificial insemination (AI) programs, purchase of only virgin bulls and heifers, and vaccination. Vaccination is an important component of any prevention program as it has been shown to reduce pregnancy losses associated with *T foetus* infection in beef herds [2,17–20]. The use of commercially available vaccines based on whole-killed protozoa alone or in combination with *Campylobacter* and *Leptospira* in cows naturally mated with *T foetus*–infected bulls have been proven to induce a protective immune response, shorten genital infections, and reduce reproductive failure due to *T foetus* [2,17–20]. Moreover, a major *T foetus* immunoaffinity-purified surface antigen containing a lipophosphoglycan (LPG)-protein complex has been used for experimental immunization in cattle [10,12,13,15]. In those studies, systemic or vaginal administration of the surface antigen TF1.17 to nonpregnant females was able to protect cattle from bovine trichomoniasis *via* induction of both IgG1 and IgA antibodies in genital secretions and IgG1 antibodies in serum [10,12,13,15]. However, in those studies, heifers were challenged with *T foetus* 2 weeks after the last immunization. Thus, the duration of serum and genital IgG antibody response to vaccination with *T foetus* was not determined. Additionally, TF1.17 antigen is partially shed from the trichomonad surface. Since the commercial *T foetus* vaccine is composed of killed whole-cell antigen, it has not been determined whether an adequate response to this surface/shed antigen is stimulated.

In the present study, we tested the hypothesis that administration of a commercial vaccine containing killed whole *T foetus* provides a significant induction of specific IgG response to both killed whole-cell *T foetus* antigen and to *T foetus* TF1.17 surface antigen in serum, vaginal secretions, and uterine flush samples of beef cows, and that the levels of IgG remain elevated over baseline for several weeks.

2. Materials and methods

2.1. Experimental design and animal husbandry

This study was designed as a randomized controlled trial. The study was performed at the University of Georgia Rose Creek Farm in the Oconee County, Georgia. Twenty clinically normal postpartum (64.8 ± 16.7 days postpartum) beef cows were utilized in this trial. Cows were sourced from a commercial herd that had an adequate biosecurity program in place, to which no new cattle had been introduced in the previous 12 months and had no history of venereal diseases. The cows and their calves were housed in a 16-acre pasture with adequate shade, mineral supplementation, and water *ad libitum*. The reproductive program of the herd corresponds with a 3-month breeding season using artificial insemination. Additionally, 21 days after AI, the cows are placed with two *T foetus*–negative satisfactory potential breeding Angus bulls in the same pasture for natural mating. Before initiating the study, vaginal secretions were collected from each cow using plastic-infusion pipettes coupled to 12-mL syringes and submitted to the University of Georgia, College of Veterinary Medicine, Athens Veterinary Diagnostic Laboratory, Athens, GA, USA, for *T foetus* diagnosis *via* culture and polymerase chain reaction (PCR). In addition, preputial scraping samples were collected using the same collection system from the two bulls used for natural mating in the previous breeding season for *T foetus* diagnosis at the Athens Veterinary Diagnostic Laboratory. These samples were immediately cultured using a special media for *T foetus* (Inpouch TF medium; Biomed Diagnostics, White City, OR, USA), incubated at 37 °C, and examined daily for a week by light microscopy and submitted to PCR analysis. All animals were *T foetus* negative by PCR and culture tests. The research protocol was reviewed and approved by the University of Georgia, Institutional Animal Care and Use Committee (protocol number A2013 03–018-Y3-A0).

2.2. Estrus cycle synchronization

Cows received 25 mg of PGF2α (Lutalyse, Zoetis Animal Health, Florham Park, NJ, USA) intramuscular on Day 7 to standardize the stage of their estrus cycle eliminating a