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Short communication

Systemic humoral immunity in beef bulls following therapeutic vaccination against *Tritrichomonas foetus*

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ABSTRACT

The utility of therapeutic vaccination of bulls against *Tritrichomonas foetus* has been advocated in previous studies, but anecdotal reports suggest this practice does not clear infections and may additionally confound diagnostic testing by reducing parasite burdens below detectable limits. The objective of this study was to characterize the systemic humoral immune response to therapeutic vaccination in *T. foetus*-infected bulls over a period of four months using an indirect ELISA and to compare the dynamics of this response to culture and PCR results to establish the existence of a relationship (or lack thereof) between immunization and infection status. A study population of 4- to 6-year-old *T. foetus*-infected beef bulls ($n = 20$) was divided equally into a treatment group and a control group. The treatment group received two doses of commercially prepared whole cell killed vaccine 2 weeks apart while the control group received injections of vaccine diluent. Blood samples were collected at each injection and at 4 subsequent dates every 4 weeks thereafter (i.e. 0, 2, 6, 10, 14, and 18 wks) to measure IgG_1 and IgG_2 antibody subisotype response via an indirect ELISA. Preputial smegma samples were collected at the four monthly intervals following vaccination for diagnosis of infection via InPouch™ culture, Modified Diamond's Medium (MDM) culture, and PCR. Humoral response for both IgG isotypes from week 2 through week 18 were significantly increased in vaccinates compared to controls. No significant decrease in infection prevalence was detected in the treatment group for any of the diagnostic methods used. The apparent lack of pathogen clearance during a stimulated immune response suggests that therapeutic vaccination may not be a useful *T. foetus* management practice.

1. Introduction

The protozoan parasite *Tritrichomonas foetus* is a major challenge to cattle health and efficient beef reproductive performance worldwide. Reproductive tract infection by *T. foetus* is most deleterious to the adult cow, in which signs can range from mild vaginitis to more serious complications such as pyometra, early embryonic death, and abortion. The bull is considered to be an asymptomatic carrier harboring parasites in the crypts of its preputial epithelium for multiple breeding seasons (Pereira-Neves et al., 2011). In this way, bulls serve as an important reservoir for *T. foetus*, and unidentified bull or cow 'carriers' may contribute to problems of persistent infection and associated production losses (Rae & Crews, 2006).

Because no effective treatment for *T. foetus* infection has been

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sanctioned for use in the United States (Michi et al. 2016), immunization remains a common mode of preventative management ancillary to biosecurity measures and test-and-cull programs (Baltzell et al., 2013; Jin et al., 2014). A whole-cell, killed *T. foetus* preparation in oil adjuvant (Trichguard®, Boehringer Ingelheim Vetmedica Inc.) is USDA-approved "for use in healthy cattle as an aid in the reduction of shedding of *Tritrichomonas foetus*" in the United States (Boehringer Ingelheim Vetmedica Inc., 2018). While vaccinated cows are still susceptible to infection, the duration and severity of their clinical signs may be markedly reduced (Rae & Crews, 2006; Cobo et al., 2010). The benefit of vaccination in bulls has been debated (Rae & Crews, 2006), but evidence has suggested that vaccinated bulls challenged with preputial *T. foetus* inoculation tested negative for the parasite by both culture and polymerase chain reaction (PCR) methods 6 weeks post-challenge (Cobo et al., 2010). The study proposed that IgG_1 and IgG_2, whose serum concentrations were the highest of antibody isotypes measured in vaccinated bulls, are likely the most important elements of the systemic adaptive immune response to this pathogen and may facilitate its clearance by inhibition of trichomonad adhesion to the preputial epithelium and activation of neutrophils. Systemic IgE production may also facilitate this process by sensitizing mast cells, whose vasoactive degranulation products promote vasodilation and increased endothelial permeability that permits greater IgG translocation to the preputial mucosa (Cobo et al. 2010). IgA-containing plasma cells localized within the preputial subepithelium have been shown to proliferate in response to vaccination, but the quantity of IgA antibodies recovered from preputial secretions did not vary significantly between vaccinates and unvaccinates following challenge with the parasite as might be expected for a local, mucosal infection (Cobo et al., 2010). These findings in bulls contrast with previous evidence that mucosal IgA production works in conjunction with IgG to clear *T. foetus* from the female reproductive tract (Corbeil et al., 1998).

Though *T. foetus* vaccination has historically been prophylactic in nature, therapeutic vaccination has also been investigated. A recent critical review (Baltzell et al. 2013) acknowledged the dearth of information regarding the efficacy of therapeutic vaccination in *T. foetus*-infected bulls with an adjuvanted whole cell preparation; to our knowledge, only one such study has been conducted to date (Clark et al., 1983), the results of which did not include an analysis of antibody kinetics. Anecdotal reports from beef cattle producers and veterinarians in south Florida suggest that therapeutic vaccination depresses *T. foetus* burdens below the detectable limits of culture and PCR testing without fully clearing infection, thus confounding interpretation of these standard diagnostic techniques and permitting the continued dissemination of *T. foetus* within affected herds. Such reports contradict published results suggesting that most therapeutically immunized bulls clear their original infection, although efficacy may be greater using purified membrane glycoprotein preparations (Clark et al., 1984) than using whole cell preparations (Clark et al., 1983). Clearly, the utility of therapeutic vaccination must be established to avoid undue financial input by beef cattle producers if such a practice proves unlikely to yield a reasonable return on investment.

The objective of this study was to characterize the systemic humoral immune response to therapeutic vaccination in *T. foetus*-infected bulls over a period of four months and to compare the dynamics of this response to culture and PCR results to establish the existence of a relationship (or lack thereof) between therapeutic immunization and infection status. We hypothesized that IgG_1 and IgG_2 serum level would increase above pre-vaccination levels for at least 16 weeks, based on previous evidence that therapeutically immunized bulls that have cleared their initial infection and are re-challenged with preputial *T. foetus* inoculation at this time point do not develop new infections (Clark et al., 1984), although the likely role of B-cell memory in this apparent resistance should not be disregarded. We also hypothesized that positive infection status amongst therapeutically vaccinated bulls would decrease significantly over the course of the study (as verified by culture and PCR diagnostic techniques) due to the efficacy of prior therapeutic vaccination studies (Clark et al., 1983; Clark et al., 1984).

2. Materials studied, area descriptions, methods, and techniques

2.1. Bull treatment groups

Twenty *T. foetus*-infected bulls between the ages of 4 to 6 years were acquired from ranches in south-central Florida. Angus (n = 10), Brangus (n = 3), Braford (n = 3), Charolais (n = 2), and Holstein (n = 2) breeds were represented. A positive infection status was defined by and confirmed only when both InPouch culture and PCR diagnostic tests were positive. Bulls were kept on pasture at Buck Island's MacArthur Agro-Ecology Research Center (Venus, FL) during the spring of 2014. Two groups of 10 animals were randomly assigned to treatment and negative control groups, which were then isolated from one another. One group of 10 (treatment) was administered a commercial whole cell killed *T. foetus* vaccine (Monovalent Trichguard®, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) following label instructions (i.e. two 2 mL subcutaneous injections given 2 weeks apart). The second group (control) received the vaccine diluent/adjuvant, also via subcutaneous injection, as per the treatment group. A single *T. foetus-infected* bull vaccinated according to the schedule listed above was included in the experimental population to serve as a positive control in the subsequent ELISA assays.

2.2. Bull serum processing

At each immunization (week 0 and week 2) and at 4 monthly intervals (weeks 6, 10, 14 and 18) post-vaccination, blood was collected from all bulls by either jugular or coccygeal venipuncture using vacutainer tubes without anticoagulant. Filled tubes were left at room temperature (25 °C) until a visible clot reaction occurred. Blood samples were transported back to the lab on ice. The tubes were then centrifuged at 1800 × *g* for 10 min. The sequestered serum fractions were transferred to conical tubes that were subsequently placed into storage at −80 °C for use in future assays.

2.3. Preputial sampling

At four monthly intervals post-vaccination, smegma samples were collected from each bull via preputial scraping and aspiration using a Perspex artificial insemination pipette. Samples were immediately transferred to both an InPouch™ TF culture (BioMed Diagnostics, Inc., White City, OR) and a pre-prepared 5 mL vial of Diamond's trypticase-yeast extract maltose (TYM) medium without agar (Diamond, 1983). Both cultures were incubated at 37 °C with microscopic examination subsequently performed every 24 h for 3 consecutive days to inspect for signs of trichomonad colony growth (i.e. characteristic erratic spiral movements and morphological characteristics consistent with *T. foetus* trophozoites). Samples were declared culture positive upon confirmation of trichomonad growth at all time points during incubation, whereas samples were declared culture negative if no trichomonad growth was observed. After incubation, medium from each InPouch™ culture was tested by quantitative PCR (qPCR) at the Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX and assigned either a "positive" or "negative" infection status according to their Ct value. Samples with a Ct value below 35 were interpreted as "positive". Samples with a Ct value greater than 35.1 or an "undetected" classification were interpreted as "negative". Samples that gave Ct value between 35.1 to 40 were repeat tested; if the repeat test yielded a Ct value above 35.1, they were interpreted as negative. Aliquots were stored at −20 °C before shipment.