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Comparative histopathology and antibody responses of non-*Tritrichomonas foetus* trichomonad and *Tritrichomonas foetus* genital infections in virgin heifers

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Abstract

The potential pathogenicity of non-*Tritrichomonas foetus* trichomonads (NTfTs) recently isolated from the prepuce of virgin bulls is not known. The purpose of this study was to determine the ability of these NTfTs to cause disease in the female reproductive tract relative to *T. foetus*. Forty-four virgin heifers were experimentally infected intravaginally with either one of two NTfTs (*Pentatrichomonas hominis* or *Tetratrichomonas* spp.), *T. foetus*, or sterile media and cultured weekly from 0 time until slaughter at 8 weeks. Serum and vaginal antibody responses during infection were assessed, and the reproductive tracts were histologically examined, scored, and compared based on numbers of neutrophils, eosinophils, lymphocytes, and plasma cells as well as the qualitative appearance of the reproductive tract. The NTfTs did not persist in the reproductive tract, while *T. foetus* persisted for at least 6–8 weeks. Further, no vaginal IgA response to infection was found in NTfT-infected and control heifers, but a vaginal IgA response was present in the *T. foetus*-infected group. Heifers infected with NTfT or controls showed little mucosal inflammatory response compared to *T. foetus*-infected heifers. Among the trichomonads studied, persistent infection by *T. foetus* alone seems responsible for uterine inflammatory lesions usually associated with pregnancy loss. The NTfTs studied in this work only transiently infected the vagina and were associated with strictly mild inflammatory changes, which probably do not cause significant disease, i.e., pregnancy loss.

Keywords: *Tritrichomonas foetus*; *Tetratrichomonas* spp.; *Pentatrichomonas hominis*; Pathogenicity; Virulence; Non-*Tritrichomonas foetus* trichomonads; NTfTs; Experimental infection

1. Introduction

Trichomoniasis is a venereal disease of cattle, caused by the flagellated protozoan, *Tritrichomonas foetus*. This parasite is responsible for widespread pregnancy loss, and occasional post-coital pyometra in infected females, and is thus an economically important pathogen (BonDurant and Honigberg, 1994). The culling of valuable bulls infected with the protozoan is also a

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major source of economic loss, as there is currently no legal and efficacious treatment in the U.S. Recently, there has been some confusion of identity between *T. foetus* and other trichomonads isolated from the prepuce of virgin bulls (BonDurant et al., 1999). Using only culture and morphologic identification of live organisms by light microscopy, diagnostic laboratories and practitioners incorrectly identified virgin bulls as infected with *T. foetus*, requiring ranchers to unnecessarily remove these bulls from breeding. Many of these cultures were later identified as non-*T. foetus* trichomonads (NTfTs), using polymerase chain reaction (PCR) (Grahn et al., 2005). *Tetratrichomonas* spp. and *Pentatrichomonas hominis* were the most frequently identified NTfT organisms in virgin bulls (Hayes et al., 2003). Although NTfTs appear to be common in bulls, the potential pathogenicity of NTfTs in the female has not been investigated. This study compares the immunologic and histopathologic changes in the reproductive tract of heifers inoculated with *P. hominis*, or *Tetratrichomonas* spp. with *T. foetus*-infected and uninoculated heifers with the aim of determining the potential pathogenicity of NTfTs in cattle.

2. Materials and methods

2.1. Preparation of infectious inoculum

A *Tetratrichomonas* spp. isolated from the prepuce of a bull in 2001 (Accession number 2001-0006 from Montana Department of Livestock Diagnostic Laboratory, Bozeman, MT) was cultured and maintained in Schneider's egg shell medium (Schneider, 1942). *P. hominis* was acquired from a diagnostic laboratory case of a genital infection (Accession number 2002-0082 from California Animal Health and Food Safety Laboratory necropsy number D02-8721) and was also maintained in Schneider's egg shell medium. The positive control organism, *T. foetus* D1, was originally obtained from a cow with severe post-coital pyometra from a beef herd with infertility (Skirrow and BonDurant, 1990b). Stabilates of this organism were used in several studies to successfully infect 100% of experimental heifers at a dose of 1×10^6 *T. foetus* organisms intravaginally at estrus (BonDurant et al., 1993). Cultures of *T. foetus* D1 were maintained using trypticase–yeast extract–maltose (TYM) medium containing 10% fetal calf serum (Diamond, 1983).

2.2. Experimental subject cattle

This study was conducted over 2 years, using 44 cross-bred Angus post-pubertal virgin heifers, 20 heifers in the first year (experiment A) and 24 heifers in the second year (experiment B). A third group of nine Angus or cross-bred Angus heifers that were never vaginally inoculated and that were sampled only once at the time of slaughter were used as uninoculated controls. The ELISA data from each experiment is reported separately to account for differences by year. All heifers were approximately 13–15 months old, 450–570 kg at slaughter, and were acquired from a university test station. All procedures and animal handling were in accordance with Institutional Animal Care and Use Committee guidelines.

The heifers were maintained in a feedlot during the experiment, given water *ad libitum*, and fed mixed alfalfa hay and grain at a rate calculated to allow a weight gain of approximately 1.2 lbs (0.55 kg) per heifer per day. Each animal was restrained in a hydraulic chute once a week for examination and sampling of blood and cervical vaginal mucus (CVM). Heifers were initially transrectally palpated to confirm their non-pregnant status, and estrous cycles were synchronized using two intramuscular injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Lutalyse®, 25 mg, Pfizer, Pharmacia-Upjohn, Kalamazoo, MI, USA) 2 weeks apart and one injection of GnRH (Cystorelin®, 100 μg IM, Merial, Duluth, GA, USA) 2 days after the second $PGF_{2\alpha}$ injection. Eight weeks after inoculation, they were slaughtered at a commercial abattoir where the reproductive tracts were removed, immediately cultured, cooled, and transported to the laboratory for histologic sampling within 2 h of slaughter.

2.3. Experimental inoculations

The heifers were divided into six treatment groups: (i) 10 heifers inoculated intravaginally with 1×10^6 *P. hominis* (six in experiment A and four in experiment B); (ii) four heifers inoculated intravaginally with 1×10^8 *P. hominis* (all four in experiment B); (iii) 10 heifers inoculated with 1×10^6 *Tetratrichomonas* spp. (six in experiment A and four in experiment B); (iv) four heifers inoculated with 1×10^8 *Tetratrichomonas* spp. (all four in experiment B); (v) eight inoculated with 1×10^6 *T. foetus* (four in experiments A and B); and (vi) eight inoculated with sterile media (TYM) (four in experiment A and four in experiment B). In addition, a procedural control group of nine virgin heifers was included that was neither inoculated nor sampled before slaughter.

2.4. Sampling

For 1 week before inoculation, and weekly for 8 weeks thereafter, 10 ml of blood was collected in a