Failure of a novel surface polysaccharide targeting vaccine to prevent *Tritrichomonas foetus* infection in beef cattle

T. B. Hairgrove, DVM, DABVP[1]; **J. A. Thompson**, DVM, DVSc, DACT, DACVPM[2]; **J. N. Rocha**, PhD[2]; **M. Vinacur**, BA[3]; **C. Roberts**, BSc[1]; **G. B. Pier**, PhD[3]; **C. Cywes-Bentley**, PhD[3]
[1]Texas A&M AgriLife Extension Service, 241 D Kleberg TAMU 2471, College Station, TX 77843-2471
[2]College of Veterinary Medicine and Biomedical Science, Texas A&M University College Station, TX 77843-4475
[3]Division of Infectious Diseases, Dept Medicine, Brigham and Women's Hospital, Harvard Medical School, 181 Longwood Ave, Boston, MA 02115

Introduction

Tritrichomonas foetus (*T. foetus*) is the causative agent of bovine trichomoniasis that has a major impact on production costs for beef cattle farmers. Immunization strategies to effectively protect against *T. foetus* are a high priority. *T. foetus* expresses a surface polysaccharide, beta 1-6 poly-Nacetyl glucosamine (PNAG). A PNAG-specific vaccine has demonstrated protection in pigs and horses. This study attempted to protect pregnant cows from an experimental *T. foetus* infection by prior vaccination with a PNAG-specific vaccine.

Materials and Methods

PNAG expression by *T. foetus* clinical isolates was assessed by immunofluorescence confocal microscopy using a labeled PNAG-specific monoclonal antibody. Cows were vaccinated in the neck subcutaneously with 200 µg of a synthetic oligosaccharide of PNAG composed of pentamers of β 1→6-linked glucosamine conjugated to tetanus toxoid (AV0328, Alopexx Vaccines) with 100 µl of Specol adjuvant, twice, 2 weeks apart. PNAG antibody titers were assessed by ELISA on d 0 and 35 post-immunization. PNAG antibody functional activity was assessed by complement component C1q deposition, bactericidal or opsonophagocytic killing assays. Heifer estrus cycles were synchronized using 3 doses of 25 mg of dinoprost tromethamine (Estrumate™) at 14-d intervals, prior to insemination with fertile frozen/thawed semen and inoculation with 106 *T. foetus* organisms into the anterior vagina beneath the external cervical os, followed by 100 mcg gonadorelin (Facrel™). Culture and pregnancy testing were performed in a blinded fashion. Vaginal mucus was evaluated weekly for infection status from d 4 until d 32 post-insemination. The mucus was immediately inoculated into an Inpouch™ container and cultured at 98.6°F (37°C). On d 32 post-insemination, pregnancy was evaluated by transrectal ultrasonography. From d 32 until 4 months post-insemination, pregnancy evaluations were repeated and vaginal cultures collected at monthly intervals. *T. foetus* was identified by morphology and motility on d 1 to 4 and d 7. Fisher's Exact Test compared proportions of infected and non-infected animals in the control and vaccine groups.

Results

PNAG-specific antibody titers were achieved after vaccination. Fifteen of 16 heifers remained vaginal culture-positive until d 32 post-insemination. On d 60, 14 of 16 heifers were culture-positive and on d 90, 5 of 16 heifers were culture-positive. At the study conclusion (d 120), 3 of 16 animals were culture positive, including 1 control pregnant cow and 2 vaccinated non-pregnant cows. The rates of positive culture were not significantly different between vaccinates and controls ($P > 0.10$). At d 32 post-insemination, 3 control heifers and 2 vaccinated cows were pregnant. One vaccinated cow aborted before 4 months of gestation, leaving 3 control pregnant heifers and 1 vaccinated pregnant heifer at the study end. Pregnancy rates were not significantly different between groups ($P > 0.10$). Further *in vitro* assays determined antibodies elicited were also non-protective in bacterial killing assays.

Significance

The study illustrates considerable potential for the development of a novel vaccine for *T. foetus*. First, unlike bulls, cows do clear the infection and, presumably, the immune system plays an important role. With this study's experimental challenge, only 1 of 16 cows appeared to clear the (vaginal) infection before embryonic signaling (approximately d 18) and before placental attachment (approximately d 32). The challenge dose of 106 organisms may be a factor and further studies should include a vaccination trial using natural insemination by infected bulls. The current study also showed that vaginal infection is often tolerated during pregnancy, but abortion did not occur. In contrast to PNAG and Specol vaccination of pigs and horses, that elicited functional and protective antibodies against other pathogens, in cows this same formulation elicited non-protective and non-functional antibodies.