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Research paper

Safety and serologic response to a *Haemonchus contortus* vaccine in alpacas

Grace VanHoy[a], Michelle Carman[b], Greg Habing[b], Jeffrey Lakritz[a], C. Austin Hinds[a], Andrew Niehaus[a], Ray M. Kaplan[c], Antoinette E. Marsh[b,*]

[a] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, OH, 43210, United States
[b] Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University, Columbus, OH, 43210, United States
[c] Department of Infectious Disease, College of Veterinary Medicine, University of Georgia, Athens, GA, 30602, United States

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ABSTRACT

Haemonchosis in camelids remains a challenging disease to treat, and prevention has become increasingly problematic due to widespread anthelmintic resistance. Barbervax®is an adjuvanted vaccine containing natural H-11, H-gal-GP antigens obtained from *Haemonchus contortus* adults via a proprietary process and solubilized in Quil A. This vaccine is approved for use in Australia, after demonstrating its safety and efficacy in sheep and goats. There are no published studies evaluating Barbervax in other ruminants/pseudoruminants such as camelids which can be parasitized with *H. contortus*. The vaccine utilizes a mixture of the parasite gut mucosal membrane enzymes including H-gal-GP and H11, involved in digesting a blood meal from the host. This study monitored the safety profile of the Barbervax® vaccine in a group of adolescent alpacas. Although designed into the original study of vaccine efficacy, the experimental infection with viable *H. contortus* third stage larvae could not be completed due to lack of detectable significant variation of infection following experimental challenge. Twelve alpacas (158 ± 15 days) were randomized to vaccination with Barbervax® or no treatment. Three doses of Barbervax® were administered at 3 week intervals and investigators involved in animal monitoring and sample collection were blinded to the groupings. Clinical pathologic parameters were evaluated 7 days before vaccination, and 1 and 2 months post-vaccination. Daily clinical observations were made and specific observations regarding the injection site and rectal temperatures were monitored in each alpaca twice daily for 1 week following vaccination. Fecal egg counts, packed cell volume, and total protein were monitored following challenge with 1500 *H. contortus* larvae on days 42, 46, and 50. An increase in rectal temperature for a duration of 2 days (range 2–4 days) was observed post-vaccination. Vaccinated alpacas were lethargic for 2-3 days following vaccination; however, they maintained an appetite and no visible or palpable injection site reactions were observed. Following the first vaccination, all animals maintained normal clinical pathologic parameters throughout the study period. The vaccinated animals did develop titers to the *H. contortus* antigen as measured by ELISA. In conclusion, the Barbervax® vaccine demonstrated safety in this small group of young, healthy alpacas, but additional studies are required to evaluate the efficacy of the vaccine under field conditions in protecting alpacas against infection with *H. contortus*.

1. Introduction

Haemonchus contortus (Barberpole worm) is one of the most significant current challenges facing the viability of small ruminant industries globally (Garner-Paulin, 2007; Jabbar et al., 2013). Haemonchosis causes clinical signs referable to anemia caused by the blood-feeding activity of the nematode and is associated with lethargy, gastroenteritis, poor nutrient uptake, clinically evident ill-thrift, and poor fiber quality (Leguia, 1991). Camelids presenting to the Hospital for Farm Animals (The Ohio State University) with anemia associated with patent *H. contortus* infection are often severely compromised by the anemia, with tachycardia, dehydration, anorexia, reluctance to stand, hypophosphatemia, hypovitaminosis D, and very low serum iron (J. Lakritz personal communication).

Due to widespread drug resistance, limited genetic pool within the camelid industry in the United States, and other factors, *H. contortus* infections significantly impact the long-term health and productivity of camelids and small ruminants. Current treatment protocols rely on repeated use of anthelmintics but widespread, multi-drug resistance to anthelmintics make treatment challenging (Howell et al., 2008; Vidyashankar et al., 2012). In spite of new drug therapies marketed outside of the USA, resistance to newer drugs is now documented

* Corresponding author at: Department of Veterinary Preventive Medicine, Ohio State University, A192 Sisson Hall, 1920 Coffey Road, Columbus, Ohio, 43210, United States.
E-mail address: marsh.2061@osu.edu (A.E. Marsh).

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(within 2 years of drug approval) (Besier et al., 2012; Van den Brom et al., 2015). The production of vaccines that augment the animal's resistance to *H. contortus,* thereby interrupting the parasite's biology, show promise (Bassetto et al., 2014). The exploited vaccine antigens were the H11 and H-gal-GP protein complexes found within the parasite's intestinal mucosa involved in the digestion of blood meals taken up by *H. contortus*. These nematode antigens reside on the mucosal membrane of the parasite's gastrointestinal tract and as such, are not available to immunize the host to these antigens during parasite infection. The vaccine antigen immunizes the host to these worm antigens so when worm blood feeding occurs, the ingested antigen specific antibodies binds to the worm's functional proteins on the brush border of its intestinal cells and compromises its digestive processes, leading to worm starvation, loss of fecundity and eventual detachment (Smith and Zarlenga, 2006). Because these antigens are "hidden" to the host's immune responses, there is no significant anamnestic response achieved in natural infections (LeJambre et al., 2008).

Sheep and goat antibodies to the H-gal-GP complex neutralize the function of this multi-protease complex and reduce the adult parasite's ability to survive. To date, this technology lacks objective evaluation in camelids. If this technology meets these two criteria, 1) safe in camelids and 2) stimulates antibodies specific to *H. contortus* H-gal-GP complex which disrupts the parasite's feeding, the vaccine could provide an adjunctive option for the control of haemonchosis, thereby limiting the use of anthelmintics. Animals in prior studies still become infected with *H. contortus*, but the vaccine has been associated with decreased fecal egg shedding, decreased pasture contamination and decreased total worm burdens (Besier et al., 2012; Bassetto et al., 2014; Besier et al., 2012; Meier et al., 2016). The *Haemonchus* vaccine (Barbervax®) is receiving new attention because of several recent breakthroughs in its research: 1) discovery that native antigen in lower concentrations will stimulate protective immunity and 2) improvements in parasite antigen production, allowing for commercially viable yields for vaccine production (Daley et al., 2010; Bassetto et al., 2014).

Evaluation of the humoral immune response in alpacas vaccinated with other vaccines (West Nile, Bluetongue and *Clostridium perfringens*) suggest that alpacas respond with a predictable humoral response in comparison to other species (Bentancor et al., 2009; Muyldermans et al., 2009; Zanolari et al., 2010). These studies have shown that antibody titers measured post-vaccination are correlated with decreased disease in these populations (Bentancor et al., 2009; Muyldermans et al., 2009; Zanolari et al., 2010). One of the drawbacks of the Barbervax® vaccine is the potential lack of anamnestic response when the alpaca acquires *H. contortus* parasites through grazing on infected pastures. Our results demonstrated the vaccine's safety in a small number of alpacas, and its ability to cause significant antibody titers in alpacas. However, without the successful establishment of the experimental challenge infection providing measurable differences in eggs per gram (EPG) or clinical parameters, the study results cannot address the vaccine's efficacy to lessen clinical disease in alpacas associated with *H. contortus* infections.

2. Materials and methods

2.1. Alpacas

A randomized, preventive challenge trial was performed with 12 young alpacas (Sargeant et al., 2010). The Ohio State University's Institutional Animal Care and Use Committee approved all animal husbandry and experimentation protocols (2016-5). Fig. 1 shows the timeline of protocol activities and Fig. 2 diagrams the experimental design. Twelve weaned alpacas: seven intact males (5 Suri, 2 Huacaya), five females (4 Huacaya, 1 Suri) between 4–6 months of age (Table 1) were acquired from a local alpaca farm. This farm was chosen as a source of animals for this study since they maintain precise records on their animals, routinely perform fecals on their individual animals and deworm animals based upon the advice of their farm veterinarian. The 12 animals chosen for study were selected based upon FEC prior to onset of study, age, and farm management scheme. The alpacas were housed at the Hospital for Farm Animals at The Ohio State University Veterinary Medical Center. Upon acquisition, physical examination, body weight, fecal, and blood samples were taken to evaluate the health status of each alpaca.

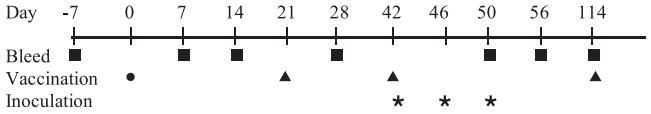

Fig. 1. Alpaca experiment timeline. Alpacas were monitored for evidence of serum antibody detection by obtaining blood samples at various time points during the course of the study (■). For alpacas that received the vaccine (n = 7), the vaccine dose was re-administered 21 days, 42 days, and 114 days (▲) after initial vaccination on day 0 (•). All alpacas were inoculated on days 42, 46, and 50 (*) via nasogastric route with viable third stage larvae (L3) obtained from an experimentally infected *Haemonchus contortus* goat. Blood samples occurred every week and fecal sampling biweekly for all alpacas following the final larval inoculation until end of the study.

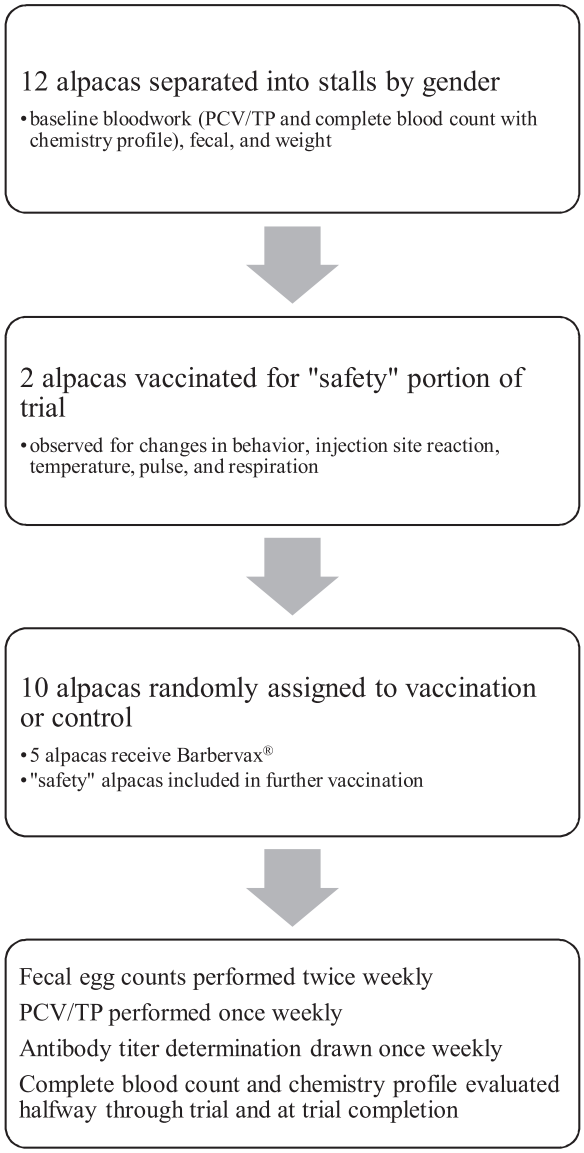

Fig. 2. Experimental design flow chart.