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Short communication

Equine antibody response to larval *Parascaris equorum* excretory-secretory products

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ABSTRACT

Parascaris equorum is an intestinal nematode of foals and young horses that can produce mild to severe pathology. Current diagnosis is limited to detection of patent infections, when parasite eggs are identified during fecal examinations. This study examined the use of larval *P. equorum* excretory-secretory (ES) products in a western blot test for diagnosis of prepatent equine *P. equorum* infection. Sera from adult mares negative for patent *P. equorum* infections, foals prior to consuming colostrum, and *P. equorum* infected foals were used as controls in this study. Study samples included sera from 18 broodmares prior to parturition and sera from their foals throughout the process of natural infection. Sera from study horses were examined for IgG(T) antibody recognition of ES products. Foals naturally infected with *P. equorum* possessed IgG(T) antibodies against 19 kDa, 22 kDa, 26 kDa, and 34 kDa ES products. However, passive transfer of colostral antibodies from mares was shown to preclude the use of the crude larval ES product-based western blot test for diagnosis of prepatent *P. equorum* infections in foals.

1. Introduction

Parascaris equorum is a nematode that commonly infects foals and yearling equids. Transmission occurs when eggs are shed in the feces of an infected equid, the eggs embryonate, and a susceptible equid ingests the infective eggs. Once infective eggs are ingested, larvae hatch in the small intestine, burrow into the wall of the gut, and undergo hepato-tracheal migration (via hepato-portal venous blood stream to the liver and lungs) (Clayton and Duncan, 1979; Srihakim and Swerczek, 1978). Larvae leave the alveoli, migrate to the pharynx and are swallowed, and then return to the small intestine where they mature and reproduce (Clayton and Duncan, 1979). During migration, larvae can cause pathology in the liver and lungs, along with respiratory signs (Srihakim and Swerczek, 1978). Large worm burdens in the small intestine can lead to weight loss, depression, and even intestinal blockage or rupture (Clayton, 1978). Shedding of *P. equorum* eggs does not begin until 10–15 weeks after initial infection (Clayton and Duncan, 1978; Lyons et al., 1976).

Equids can be diagnosed using microscopy to identify *P. equorum* eggs in feces (copromicroscopy). Recently, ultrasonography has also shown potential for diagnosis of mature ascarids within the small intestine (Nielsen et al., 2015). However, diagnosis during the prepatent period is not currently possible and copromicroscopic methods do not provide an accurate depiction of the total worm burden in the small intestine (Nielsen et al., 2010). Therefore, it becomes problematic to use current diagnostic methods for making treatment recommendations or evaluating the efficacy of anthelmintics. Furthermore, with concerns of *P. equorum* resistance to multiple classes of anthelmintics (Matthews, 2014), it is increasingly important to expand options for surveillance so that treatment frequencies can be reduced without adverse effects on equine health. The development of new serological diagnostic tools could allow for detection during the prepatent period, and provide new information on the host immune response to this parasite.

Excretory-secretory (ES) products collected from parasites *in vitro* have been used in the development of serological tests for detection of infection or larva migrans by *Toxocara canis* (de Savigny et al., 1979), *Baylisascaris procyonis* (Boyce et al., 1988; Dangoudoubiyam and Kazacos, 2009), *Ascaris suum* (Lind et al., 1993), and *Ascaris lumbricoides* (Chatterjee et al., 1996). Serological tests for evaluation of equine IgG(T) responses to parasite

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somatic or ES products have also been developed. Diagnostic potential of serological tests have been evaluated using antigens from the equine tapeworm, *Anoplocephala perfoliata* (Proudman and Trees, 1996), cyathostomes (Dowdall et al., 2002), and *Strongylus vulgaris* (Andersen et al., 2013). Sera from two foals with *P. equorum* infection were previously examined (Burk et al., 2014) and found to contain IgG(T) antibodies that recognized larval *P. equorum* ES products migrating at approximately 19 kDa, 22 kDa, 26 kDa, and 34 kDa, indicating the potential for diagnostic use.

The objectives of this study were to (1) examine IgG(T) antibody recognition of larval *P. equorum* ES products using sera from infected horses and (2) test the sera from a cohort of broodmares and their foals from birth until a period of no egg shedding.

2. Materials and methods

The University of Kentucky Institutional Animal Care and Use Committee approved all procedures used in this study (protocol #2012-0924).

Adult *P. equorum* specimens were collected from the small intestine of foals that were necropsied at the University of Kentucky's Veterinary Diagnostic Laboratory. Specimens were stored in 0.5% formalin and normal saline at 4° C. Eggs were collected using the methods of Kazacos et al. (1981). Eggs were stored, then decorticated prior to hatching as previously described (Burk et al., 2014). Eggs were mechanically hatched and larvae collected according to the methods of Urban et al. (1981). Larvae were maintained *in-vitro* for up to three weeks for collection of ES products, as described (Burk et al., 2014). Collection, concentration, and storage of ES products were performed according to the methods of Dangoudoubiyam and Kazacos (2009). The protein content of ES products was estimated using the Pierce BCA Protein Assay (Thermo Scientific, Waltham, MA).

A total of 18 Thoroughbred broodmares (mean age 8.8 years ± 4.4) kept on pasture at University of Kentucky's Maine Chance Farm (MCF) were used for this study. Foals were born between 2/29/12 and 4/7/12. Sera and ≥10 g of feces were collected from mares within one month of expected foaling date. Feces were stored at 4 °C and processed within one week after collection using a previously described modified Stoll FEC method with a sensitivity of 10 EPG (Lyons et al., 1976). Mare FEC analyses were performed at one time point for this study. However, FEC analyses for all broodmares are performed annually in March by farm staff, and mares with FEC results ≥250 strongyle-type EPG are retested in May and July. While stabled at this farm, none of the broodmares described here have tested positive for *P.equorum* eggs. Blood from each mare was collected in a 10 ml Vacutainer Serum Plus Tube (Becton, Dickinson, and Company, Franklin Lakes, NJ) and centrifuged for 10 min at 1000*g* for collection of serum. Sera were stored at −80 °C. Foal sera were processed in the same manner. Mares were designated Mare #1-18, and foals were correspondingly designated Foal #1-18, based on the order in which foals were born. The foal group consisted of 10 colts and 8 fillies.

Immediately following parturition, mare colostrum was checked using a Brix equine colostrum refractometer (Animal Reproduction Systems, Chino, CA). Four mares (Mare 6, 7, 10, and 11), had a colostrum score at or below Brix 20% (approximately 5000 mg IgG/dl or lower); consequently, their foals were supplemented with colostrum from a MCF mare with a Brix score over 20%. A veterinarian obtained a blood sample from each foal 24 h after birth to determine if the foals had adequate circulating IgG levels as determined by a glutaraldehyde coagulation test. Two foals (Foal 5 and 16) with blood IgG <800 mg/dl were given plasma provided by a plasma bank (Equine Medical Associates, Lexington, KY). Foals were pastured with their dams in individual mare-foal pairs for the first 1–2 weeks after parturition, and then were pastured with other mare-foal pairs. Foals were weaned during August 2012 and September 2012 by removing mares from pastures. Foals remained pastured for the duration of the study.

For MCF foals, ≥1 g of feces was collected either rectally or from a freshly deposited sample. Samples were stored at 4 °C and processed within one week after collection. Parasite eggs were detected using a previously described qualitative fecal flotation test using centrifugation (Lyons et al., 1988; Lyons et al., 2007). Feces were collected weekly beginning on week 8. A regular anthelmintic treatment schedule was then followed in order to maintain the farm's normal foal health care practices. Anthelmintics used included fenbendazole (10 mg/kg, Panacur; Merck Animal Health, Summit, NJ), pyrantel pamoate (6.6 mg/kg, Durvet, Blue Springs, MO), and ivermectin (0.2 mg/kg) with praziquantel (1 mg/kg) (Zimectrin Gold; Merial, Duluth, GA). By the time ivermectin with praziquantel was administered, the majority of horses had been dropped from the study after having multiple consecutive negative fecal test results. Only three horses remained on the study at this time. Two of those horses did not complete the study: Foal 15 was still shedding ascarid eggs when sold in February 2013, and Foal 13 was still shedding eggs in May 2013 when the study ended.

Additional sera from mixed-breed foals at University of Kentucky's Veterinary Science Farm (VSF) were obtained for use as positive controls (courtesy of Drs. Eugene Lyons and Martin Nielsen, University of Kentucky). Sera and manure were collected from five 3–5 month old VSF foals in July of 2011. Infection with *P. equorum* was confirmed for these foals through FEC and later that year, by necropsy. Serum from three of the foals (VSF #1, VSF #2, and VSF #3) was collected again immediately prior to necropsy. It is unknown if these foals had adequate passive transfer of antibodies.

Western blot was used to test for IgG(T) antibody recognition of *P. equorum* ES products. Procedures used for SDS-PAGE and western blot were as previously described (Burk et al., 2014). SDS-PAGE was conducted using 0.75 mm 2-D/prep 12% gels with a 4% stacking gel. The standard lane contained 2 μl Broad Range Precision Plus Protein Unstained Standards (Bio-Rad, Hercules, CA) and the sample lane contained 4 μg of *P. equorum* ES products. Equine sera for the western blots were mixed with blocking buffer in a 1/1250 dilution, and then a 1/100,000 secondary antibody dilution of HRP-labeled goat anti-horse IgG(T) (AbD Serotec, Raleigh, NC). Serum from VSF #1 was used as a control on all western blots. Serum from this foal was selected as a control because the foal was confirmed as positive for *P. equorum* by necropsy, and the serum displayed the same

Table 1
Percentage of MCF mare and foal sera with IgG(T) antibodies recognizing immunodominant *P. equorum* larval ES products.

MW (kDa)	Mare n = 18	Pre-Suckle Foal n = 18	Post-Suckle Foal n = 18	Foal upon First Positive Fecal[a] n = 18	Foal upon Second Negative Fecal[b] n = 16
19	94%	0%	100%	100%	88%
22	83%	0%	89%	89%	56%
26	89%	0%	94%	100%	63%
34	94%	0%	100%	100%	88%

[a] Between weeks 11–18, dependent upon foal.
[b] Between weeks 21–44, dependent upon foal.