Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 153 (2008) 73–84
veterinary parasitology
www.elsevier.com/locate/vetpar

Occurrence of *Anoplocephala perfoliata* infection in horses in Ontario, Canada and associations with colic and management practices

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Received 31 July 2007; received in revised form 11 January 2008; accepted 11 January 2008

Abstract

Infection with the tapeworm *Anoplocephala perfoliata* has been found to be associated with equine colic in horses in the United Kingdom. Using a matched case-control study design, data collected from 117 pairs of horses in Ontario were examined for evidence of associations between risk of colic and *A. perfoliata* infection, and between seropositivity to infection and management practices. Cases were horses in southern Ontario diagnosed with colic by local veterinarians, and control horses were from the same stables as cases and were matched by age, breed and gender where possible. Infection status was defined on the basis of positive results upon coprological examination, and/or seropositivity to a 12/13 kDa *A. perfoliata* secretory protein. Fifty-six percent of the 234 horses were seropositive for *A. perfoliata*, but eggs were found in samples from only 6% of horses. Horses dependent on pasture for a large part of their diet were significantly more likely to have ELISA optical density levels above 0.600 compared to other horses (odds ratio [OR] = 6.38; $p = 0.029$). This finding identified exposure to pasture as an important source of *A. perfoliata* infection in the horses used in the study. In a subset of 46 pairs of horses for which control horses had no known history of colic, a statistically significant negative association was found between the risk of colic and optical density (OD) levels >0.200–0.600, relative to OD levels ≤0.090 (OR = 0.08; $p = 0.017$). There was no other statistical evidence of an association between the risk of colic and *A. perfoliata* infection.

Keywords: Horse; Tapeworm; *Anoplocephala perfoliata*; Colic

1. Introduction

Anoplocephala perfoliata is an equine tapeworm of length 4–8 cm that attaches to the intestinal mucosa in the region of the ileocecal junction. This parasite has an indirect life-cycle. Eggs passed in the feces of infected horses develop in oribatid mites, the intermediate hosts. Ingestion of mites on pasture and hay facilitates completion of the cestode's life-cycle. Infection of horses by *A. perfoliata* was originally thought to be of little or no significance. However, it is now known that presence of the parasite may produce histopathological and gross lesions such as mucosal ulceration and varying grades of enteritis, with severity of lesions apparently depending on intensity of infection (Pearson et al., 1993; Nilsson et al., 1995; Rodriguez-Bertos et al., 1999;

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doi:10.1016/j.vetpar.2008.01.016

Proudman and Trees, 1999). Several clinical conditions have been associated with *A. perfoliata* infections in horses; case reports have cited infection as the possible cause of colic, intussusception and cecal rupture (Barclay et al., 1982; Proudman and Trees, 1999; Proudman and Holdstock, 2000; Ryu et al., 2001).

A number of epidemiological studies have lent support to these reports. In one such study, Proudman and Edwards collected fecal samples from 116 horses with colic admitted to The University of Liverpool Large Animal Hospital, United Kingdom (UK), and 115 non-matched controls selected from the same hospital during the same period (Proudman and Edwards, 1993). Controls were horses with no recent history of colic presenting for non-gastrointestinal problems. Fecal samples were tested for presence of *A. perfoliata* eggs using a centrifugation/flotation technique (coprological examination). No association was found between tapeworm infection and a diagnosis of colic when the latter was not analyzed by site of colic. However, analysis of the data according to anatomical site of colic revealed that the risk of ileocecal colic was significantly increased by more than three times in infected horses (odds ratio [OR] = 3.45; 95% confidence interval [CI] = 1.18–5.18).

The number of eggs excreted in the feces of tapeworm-infected animals is usually low (Slocombe, 1979), and diagnosis of *A. perfoliata* infection by centrifugation/flotation methods is reported to have a sensitivity as low as 61%, although it has been reported to be 92% in horses with >20 worms (Proudman and Edwards, 1992). Other diagnostic methods researched include the detection of coproantigen in feces (Kania and Reinemeyer, 2005), and polymerase chain reaction (Drogemuller et al., 2004). The use of antibody enzyme-linked immunosorbent assays (ELISAs) for the serodiagnosis of *A. perfoliata* infection has also been explored. Höglund et al. (1995) reported a relationship between intensity of infection and optical density (OD) results using an ELISA for detection of a scolex antigen of the parasite in equine serum. In the UK, an antibody ELISA has been developed that measures IgG(T) specific for a 12/13 kDa secretory protein of *A. perfoliata*. A prototype of this serological assay using crude excretory/secretory antigen was shown to have a sensitivity of 68% and specificity of 95% when used on 38 known *A. perfoliata*-infected ponies and 20 laboratory-reared parasite naïve animals, respectively (Proudman and Trees, 1996a). The IgG(T) assay is reported to be a reliable indicator of intensity of infection (Proudman and Trees, 1996a,b; Proudman and Trees, 1999); in another study that used sera from 72 known parasite-positive horses, the IgG(T) 12/13 kDa assay was reported to have a sensitivity of 62%, with most false negatives occurring in animals with light infections (Proudman and Trees, 1996b).

In a case-control study performed in the UK that employed the antibody ELISA, serum and fecal samples were collected from 103 horses with spasmodic colic and 103 controls matched, where possible, for age, breed, gender and stable (Proudman et al., 1998). Serum and fecal samples were also obtained from 20 horses diagnosed with ileal impaction and 40 controls also matched, where possible, on age, breed, gender and stable. Data were examined for associations between *A. perfoliata* infection, as measured by presence of tapeworm eggs in feces or ELISA OD levels, and risk of spasmodic colic or ileal impaction colic. A statistically significant positive association was found between infection with *A. perfoliata* and the risk of spasmodic colic. The odds of spasmodic colic in horses diagnosed as infected by a coprological method were 8 (95% CI = 1.54–99.0) times the odds of spasmodic colic in non-infected horses. The risk of spasmodic colic was also significantly associated with *A. perfoliata* infection as diagnosed by serological methods, with odds ratios of 3 (95% CI = 1.27–7.55), 6 (95% CI = 1.59–37.46) and 14 (95% CI = 1.64–∞) for exposures defined as serological OD levels greater than 0.200, 0.400 and 0.600, respectively. In the same study, a significantly increased risk of ileal impaction colic was also found to be associated with *A. perfoliata* infection. Odds ratios of 34 (95% CI = 4.66–∞), 26 (95% CI = 5.32–126.64) and 44 (95% CI = 7.45–∞) for exposures defined as positive fecal specimens, serum OD levels greater than 0.103, and serum OD levels greater than 0.200, respectively, were calculated. The risk of spasmodic colic also increased with increasing intensity of infection, as measured by ELISA OD levels. Using the same assay, work carried out in the Netherlands on 139 cases of colic and 139 controls matched for age and breed, in which cases were horses referred to a teaching clinic for colic of gastrointestinal origin, also demonstrated a significantly higher mean *A. perfoliata* antibody level in the colic group than in the controls (Boswinkel and van Oldruitenborgh-Oosterbaan, 2007). Furthermore, the mean antibody level in 12 horses diagnosed with ileocecal colic was higher than in controls. At necropsy, seven horses in which tapeworms were found in the ileocecal region had a significantly higher mean antibody level than that of 25 horses without tapeworms.

In a study of 78 horses with ileal impaction and 100 controls in the south-eastern USA, horses that had not

received a pyrantel salt within 3 months of admission to an equine hospital were over three times more likely to be diagnosed with ileal impaction than those that had received pyrantel (Little and Blikslager, 2002). As pyrantel was the only anthelmintic available at that time with recognized efficacy against *Anoplocephala*, this apparent protective effect was attributed to an association between tapeworms and ileal impaction. In a multicentre case-control study of horses admitted to equine hospitals in Ontario and four USA states, it was found that horses dewormed daily with pyrantel pamoate for at least 60 days during the 12-month period prior to admission had a significantly lower risk of colic than horses that had not (OR = 0.11; 95% CI = 0.02–0.55; Reeves et al., 1996). The dosage of pyrantel used was not reported. Moreover, the product was used in only two animals with colic and 11 controls. Thus, this finding should be interpreted with caution.

Several studies have shown *A. perfoliata* to be a common infection in horses, with studies in US horses reporting 52–100% of animals infected (Lyons et al., 2000; Chapman et al., 2002; Lyons et al., 2006). In work published in 1979, examination of fecal samples from 580 horses in Ontario, Canada, revealed *A. perfoliata* infection in 13.6% of the animals (Slocombe, 1979). However, there is little information on current estimates of equine tapeworm prevalence in Canada. Furthermore, it is unclear whether the association between *A. perfoliata* and colic observed in the UK is applicable to other geographic areas, as geographical variations in ecology, parasite population genetics, and equine management practices, including the use of anthelmintics, are all factors that may potentially influence the prevalence of the parasite and the number of tapeworms in horses. This, in turn, may have an effect on the ability of the parasite to produce episodes of equine colic (Slocombe, 1979; Gasser et al., 2005). The work reported here was performed with the primary objectives of investigating whether there is an association between infection with *A. perfoliata* and risk of colic in horses in Ontario, and identifying potential risk factors for exposure to *A. perfoliata*.

2. Materials and methods

2.1. Selection of study horses

Between May 2003 and August 2004, letters were sent to 60 equine veterinary practitioners in Southern Ontario explaining the objectives of the project and inviting their participation. Veterinarians were identified using databases provided by the Ontario Association of Equine Practitioners and the American Association of Equine Practitioners. The list of veterinarians invited to participate included practitioners from all segments of the horse industry. Veterinarians who agreed to participate ($n = 32$) were provided with eight sample-collection kits each, and were requested to submit fecal and blood samples from horses diagnosed with colic and from matched controls from the same farm. Colic was defined as clinical signs consistent with the presence of abdominal pain. Criteria for selection of controls were that they should, as much as possible, be of the same age, breed and gender as the colic cases with which they were matched, and should not have suffered from colic for at least 1 year prior to enrolment in the study. There were no exclusion criteria for cases or controls. Using a questionnaire, owners of study horses were asked to provide information on factors pertaining to horse demographics (age, gender and breed), horse behaviour, horse and stable management, and prior medical history.

2.2. Processing of samples

Within 24 h of collection, blood and fecal samples from the study horses were sent to the Ontario Veterinary College (OVC) by courier. There, fecal specimens were examined by a single laboratory technician for parasite eggs using the Cornell–Wisconsin method (Egwang and Slocombe, 1982). In specimens positive for *Anoplocephala* eggs, five oncospheres, if available, were measured to determine the species of *Anoplocephala* (Eckert et al., 1992).

Sera were separated from blood samples within 24 h of arrival at the OVC and stored at −70 °C. Sera were then transported to the Diagnosteq Laboratory (Liverpool, UK) for analysis with the *A. perfoliata* antibody ELISA developed by Proudman and Trees (1996b). Results of antibody ELISA testing were expressed as OD values as well as estimated infection intensity levels, categorized as follows in accordance with cut-off levels used by the laboratory: OD 0.000–0.200 = zero/low infection intensity; OD 0.201–0.600 = moderate infection intensity; OD >0.600 = high infection intensity (Proudman et al., 1998).

All laboratory personnel carrying out fecal or serological analyses were blind to the case or control status of samples.

2.3. Statistical analysis

Data were entered into a database using data entry software (EpiData, Odense, Denmark) and exported to Stata 9.0 (College Station, Texas) for statistical analysis.

Following descriptive analysis of each variable, simple logistic regression was used to verify similarity between cases and controls with respect to horse characteristics and management variables. Possible univariate associations between colic and dichotomous variables indicating tapeworm infection were then investigated by computing estimates of McNemar's chi-square and odds ratios with 95% confidence intervals for each variable, using the 'mcc' command in Stata 9.0 to account for matching. All tests were 2-sided, with a p-value of <0.05 indicating statistical significance. Estimates were based on the distribution of matched exposed and non-exposed pairs of horses. Conditional fixed effects logistic regression was employed to explore relationships between risk of colic and variables indicating infection with tapeworms.

Management variables were investigated for statistical associations with *A. perfoliata* ELISA OD levels using mixed effects linear and logistic regression with the inclusion of matched pair ID, or both matched pair and veterinarian ID, as random effects. These random effects were included to control for any effects of clustering arising within pairs matched by stable or groups of horses diagnosed by the same veterinarian, on our estimates of the measures of association and their variance. Significance of random effect terms was computed using the likelihood ratio test (Dohoo et al., 2003). Logistic mixed models were constructed using ordinary quadrature methods. Intra-class correlation coefficient estimates for logistic models were computed by the latent variable approach, in which $\rho = \sigma_h^2/(\sigma_h^2 + \pi^2/3)$, with σ_h denoting the pair random effect variance (Dohoo et al., 2003).

Model diagnostics were performed by examination of residuals, leverage values and delta–betas for evidence of outliers or undue leverage or influence on the models. For conditional logistic models, diagnostics were done on unconditional models constructed using the independent and dependent variables included in the conditional models (Dohoo et al., 2003).

3. Results

Fecal and blood samples were collected from 234 horses, comprising 117 matched pairs. Colics were cases with dates of onset from July 2003 to November 2004. Controls were enrolled in the study at the same time as cases.

3.1. Description of study horses

Ages of horses ranged from 5 months to 22 years, with a median of 7 and a mean of 8.9 years. Three horses (two colic cases and one control) were less than 1 year old, and one horse (a colic case) was 1 year of age; all other horses were over 1 year old. Information on gender was available for 187 of the 234 horses. Of these, 11 (5.9%) were stallions, 81 (43.3%) were mares, and 95 (50.8%) were geldings. Information on past history of colic was missing for some horses, but was available for 88 colic cases and 86 control horses (Table 1). The type of colic was identified in only seven cases. Of these, two were described as colitis and and five cases were described as one each of the following: testicular hernia, impaction, tumours, 'gas colic' and nephrosplenic entrapment.

In spite of efforts to enrol only control horses with no known episodes of colic within the preceding year, information provided by owners suggested that only 58 control horses met this criterion. Of the 117 controls enrolled by participating veterinarians, 48 were described as having no known history of colic and 10 were believed to have had previous episodes of colic more than 1 year prior to enrolment. Three horses were

Table 1
Summary of information on colic history for horses with colic (colic cases) and control horses

History of colic	Colic cases		Control horses		Colic cases with 'true' controls		'True' control horses	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
No known episodes of colic	42	47.7	48	55.8	25	54.4	46	100.0
One previous episode during the past year but not the past month	5	5.7	3	3.5	2	4.3	0	0.0
More than two episodes during past year	4	4.5	0	0.0	4	8.7	0	0.0
Previous episodes more than 1 year ago	16	18.2	10	11.6	10	21.7	0	0.0
Unknown	21	23.9	25	29.1	5	10.9	0	0.0
Total	88	100.0	86	100.0	46	100.0	46	100.0

Results are provided for all horses for which information on history of colic was available, as well as for a subset of 46 matched pairs of horses for which the control horses had no known history of colic ('true controls').

Table 2
Total number of anthelmintic doses received by horses with colic (colic cases) and control horses in the year preceding the study

Total number of anthelmintic doses in previous year	Colic cases		Control horses		Colic cases with 'true' controls		'True' control horses	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
1	7	10.2	6	9.0	5	14.7	4	11.7
2	13	18.8	19	28.4	3	8.8	5	14.7
3	19	27.5	10	14.9	8	23.5	6	17.7
4	14	20.3	16	23.9	7	20.6	7	20.6
5	8	11.6	9	13.4	5	14.7	7	20.6
6	8	11.6	7	10.4	6	17.7	5	14.7
Total	69	100.0	67	100.0	34	100.0	34	100.0
Missing	48	N/A	50	N/A	12	N/A	12	N/A
Grand total	117	N/A	117	N/A	46	N/A	46	N/A

Results are provided for all horses for which information on history of colic was available, as well as for a subset of 46 matched pairs of horses for which the control horses were 'true controls', i.e. had no known history of colic.

known to have had at least one episode of colic during the year prior to enrolment, and information on colic history was described as 'unknown' for 25 control animals. Veterinarians were unable to provide a complete history of colic for all horses, and due to confidentiality issues, we were unable to contact the owners for this information. Because of this, no information on colic history was available for the remaining 31 control horses. Due to concerns over the accuracy of the dates of previous episodes of colic as recalled by owners, we chose to select for analysis only those controls with no known history of colic and with complete serological data (referred to hereafter as 'true controls'; $n = 46$).

For the 46 matched pairs with 'true' controls, ages of horses ranged from 5 months to 22 years, with a median of 8 and a mean of 9.1 years. Two horses (one colic case and one control) were less than 1 year old, and one horse (a colic case) was 1 year of age; all other horses were over 1 year old. Six (6.5%) of the 92 horses were stallions, 37 (40.2%) were mares, and 49 (53.3%) were geldings.

3.2. Anthelmintic use

Information on anthelmintic use was available for 136 horses (67 controls and 69 colic cases, Table 2), all of which had received at least one anthelmintic treatment in the previous year. Of these, details of each medication used and number of doses administered were provided for 125–128. One hundred and six (84.8%) had been treated with ivermectin, 42 (33.6%) had received moxidectin, and 79 (63.2%) had received pyrantel, of which 29 horses had received a single treatment of double-dose pyrantel. Twenty-four (19.2%) had received at least one dose of an anthelmintic other than ivermectin, moxidectin or pyrantel. In most cases this was fenbendazole. However, one horse had received a single dose of an ivermectin–praziquantel combination. Tables 3a–3c summarize details of ivermectin, moxidectin and pyrantel treatment of the 46 control horses with no known history of colic, and their matched case animals.

Table 3a
Use of ivermectin in 46 colic cases and 46 matched control horses with no known history of colic

Number of treatments in previous year	Number of colic horses	Percent of colic horses	Number of control horses	Percent of control horses
0	5	14.7	2	5.9
1	9	26.5	10	29.4
2	14	41.2	15	44.1
3	5	14.7	5	14.7
4	1	2.9	2	5.9
Total	34	100.0	34	100.0
Missing	12		12	
Grand total	46		46	

Table 3b
Use of moxidectin in 46 colic cases and 46 matched control horses with no known history of colic

Number of treatments in previous year	Number of colic horses	Percent of colic horses	Number of control horses	Percent of control horses
0	23	69.7	22	64.7
1	5	15.2	7	20.6
2	4	12.1	4	11.8
3	0	0.0	0	0.0
5	1	3.0	1	2.9
Total	33	100.0	34	100.0
Missing	13		12	
Grand total	46		46	

Table 3c
Use of pyrantel in 46 colic cases and 46 matched control horses with no known history of colic

Number of treatments in previous year[a]	Number of colic horses	Percent of colic horses	Number of control horses	Percent of control horses
0	10	30.3	12	35.3
1	7	21.2	8	23.5
2	9	27.3	8	23.5
3	6	18.2	6	17.7
9	1	3.0	0	0.0
Total	33	100.0	34	100.0
Missing	13		12	
Grand total	46		46	

[a] Total number of doses, whether given as double or single dose treatments.

Eight (4.8%) of 165 horses had been tested for fecal parasite eggs in the previous year. Information on fecal testing was unavailable for the remainder of the horses.

3.3. Prevalence of intestinal parasites

3.3.1. Anoplocephala perfoliata

More than half (56.4%) of the 234 horses tested by ELISA were classified as being seropositive for *A. perfoliata,* with OD values greater than 0.200, indicating moderate or high infection intensities. Intensities of infection, as defined by ELISA OD levels of colic cases and control horses, including the subset of 46 pairs with 'true' controls, are summarized in Table 4a.

Fecal specimens from 233 horses (117 colic cases and 116 controls) were submitted for examination for intestinal parasite eggs. In contrast to results of ELISA serology, *Anoplocephala* eggs were found in samples from only 6% of the 233 horses tested (Table 4b). All oncospheres were identified as *A. perfoliata*.

3.3.2. Other gastrointestinal parasites

Strongyle eggs were detected in the feces of 42.9% of 233 horses tested (Table 5), and eggs of the equine

Table 4a
Intensity of *Anoplocephala perfoliata* infection in horses with colic (colic cases) and controls

Intensity of infection	Colic cases ($n = 117$, %)	Control horses ($n = 117$, %)	Colic cases with 'true' controls ($n = 46$, %)	'True' controls ($n = 46$, %)
Negative/low	49.2	38.8	58.7	43.5
Moderate	21.2	34.5	15.2	30.4
High	29.6	26.7	26.1	26.1
Total	100.0	100.0	100.0	100.0

Results are shown for all horses, and for the subset of 46 matched pairs for which the control horses ('true controls') had no known history of colic. Levels of infection are classified based on results of *A. perfoliata* antibody ELISA testing. (Optical density 0.000–0.200 = zero/low infection intensity; optical density 0.201–0.600 = moderate infection intensity; optical density >0.600 = high infection intensity).

Table 4b
Results of microscopic examination of fecal specimens from horses with colic (colic cases) and controls for eggs of *Anoplocephala perfoliata*, using the Cornell–Wisconsin method

Fecal egg count (level)	Colic cases ($n = 117$, %)	Control horses ($n = 116$, %)	Colic cases with 'true' controls ($n = 46$, %)	'True' controls ($n = 46$, %)
Negative	94.0	93.9	93.5	93.5
Low	6.0	5.2	6.5	4.3
Moderate	0.0	0.9	0.0	2.2
Total	100.0	100.0	100.0	100.0

Results are shown for all horses, and for the subset of 46 matched pairs for which the control horses ('true controls') had no known history of colic. For one control horse, no fecal specimen was available. Fecal egg count levels: low = 0.32–32 eggs per gram of feces; moderate = 32.3–96 eggs per gram of feces.

roundworm, *Parascaris equorum*, in fecal samples from 4.7% of 233 horses (Table 6). Overall, fecal helminth eggs (*A. perfoliata*, strongyles, *P. equorum,* or other helminths) were detected in the feces of 112 (48%) of the 233 horses tested.

3.4. Differences between case and control groups

Matching of cases and controls by age, breed and gender could not be fully achieved for all pairs of horses. However, matched controls were available for more than 90% of cases. Results of univariable logistic regression with colic or control status as the outcome revealed no statistically significant difference between colic and control horses with respect to management factors or any other variable on which colic cases and controls had been matched (e.g. age, breed and gender). This applied to all horses, as well as to the subset of 46 pairs with 'true' controls.

3.5. Association between tapeworm seropositivity/prevalence and colic

For the 46 matched pairs of horses with 'true' controls (controls with no known history of colic), the following variables were investigated for associations with risk of colic by univariable conditional logistic regression and by computing estimates of McNemar's chi-square with exact *p*-values:

- Presence or absence of fecal tapeworm eggs.
- Seropositivity to *A. perfoliata* (defined as medium or high ELISA OD levels [>0.200]).
- Seropositivity to *A. perfoliata* (defined as high ELISA OD levels only [>0.600]).
- Presence or absence of fecal strongyle eggs.
- Presence or absence of fecal roundworm eggs.
- Presence or absence of fecal helminth eggs (any).

McNemar's chi-square statistic showed none of these variables to be significantly associated with risk of colic in the subset of 46 matched pairs of horses (i.e. $p > 0.05$; Table 7). Optical density levels greater than 0.200 had a (non-significant) sparing effect on colic (OR = 0.25 [95% CI = 0.03–1.25]; $\chi^2 = 0.058$; $p = 0.109$). For OD readings greater than 0.600, no effect was seen (OR = 1.00 [95% CI = 0.30–3.34]; $\chi^2 = 1.000$; $p = 1.000$).

Investigation of the relationship between seropositivity and risk of colic using univariable conditional logistic regression gave similar results (Table 8). However, when

Table 5
Results of microscopic examination of fecal specimens from horses with colic (colic cases) and controls for strongyle eggs, using the Cornell–Wisconsin method

Fecal egg count (level)	Colic cases ($n = 117$, %)	Control horses ($n = 116$, %)	Colic cases with 'true' controls ($n = 46$, %)	'True' controls ($n = 46$, %)
Negative	59.8	54.3	65.2	60.9
Low	20.5	16.4	15.2	8.7
Moderate	11.1	21.5	13.1	19.5
High	8.6	7.8	6.5	10.9
Total	100.0	100.0	100.0	100.0

Results are shown for all horses, and for the subset of 46 matched pairs for which the control horses ('true controls') had no known history of colic. For one control horse, no fecal specimen was available. Fecal egg count levels: low = 0.32–32 eggs per gram of feces; moderate = 32.3–96 eggs per gram of feces; high = greater than 96 eggs per gram of feces.

Table 6
Results of microscopic examination of fecal specimens from horses with colic (colic cases) and controls for eggs of *Parascaris equorum*, using the Cornell–Wisconsin method

Fecal egg count (level)	Colic cases ($n = 117$, %)	Control horses ($n = 116$, %)	Colic cases with 'true' controls ($n = 46$, %)	'True' controls ($n = 46$, %)
Negative	96.6	94.0	100.0	89.1
Low	1.7	4.3	0.0	8.7
Moderate	1.7	1.7	0.0	2.2
Total	100.0	100.0	100.0	100.0

Results are shown for all horses, and for the subset of 46 matched pairs for which the control horses ('true controls') had no known history of colic. For one control horse, no fecal specimen was available. Fecal egg count levels: low = 0.32–32 eggs per gram of feces; moderate = 32.3–96 eggs per gram of feces.

OD values were broken into multiple categories, a significant sparing effect was found, for horses with OD values >0.200–0.600 compared to horses with OD values ≤0.090 (OR = 0.08 [95% CI = 0.01–0.64]; $p = 0.017$). No other variables showed a statistically significant association with the risk of colic. For each conditional model, examination of residuals, leverage values and delta–betas of corresponding unconditional models showed no evidence of poor model fit.

For comparison, similar analyses were carried out on all pairs of horses for which data for variables being examined were available ($n = 116$ pairs). In these analyses, as for the 46 pairs of horses with 'true' controls, OD levels greater than 0.200 had a (non-significant) sparing effect on colic (OR = 0.5 [95% CI = 0.22–1.08]; $\chi^2 = 3.67$; $p = 0.055$; exact McNemar's significance probability 0.080; Table 9). The direction of the association was reversed for OD readings greater than 0.600, though this association was also not statistically significant (OR = 1.29 [95% CI = 0.60–2.79]; $\chi^2 = 0.50$; $p = 0.479$; exact McNemar's significance probability = 0.597). Investigation of the relationship between seropositivity and risk of colic using conditional logistic regression also gave similar results as the subset of 46 pairs of horses with 'true' controls (Table 8). As for the subset of 46 pairs, when OD values were broken into multiple categories, a significant negative association was found for horses with OD values >0.200–0.600 compared to horses with OD values ≤0.090 (Table 8). In addition, for these 116 pairs, construction of a conditional logistic regression model where seropositivity was dichotomized (with >0.200 as the cut-off value), gave a model-based p-value that also indicated a significant negative association ($p = 0.044$). As in the analyses of the subset of 46 pairs, for each conditional model, examination of residuals, leverage values and delta–betas

Table 7
Seropositivity to *A. perfoliata* at two levles (OD >0.200 and OD >0.600), as indicated by anti-12/13/kDa IgG(T) ELISA optical density (OD) levels in 46 horses with colic and 46 matched controls with no known history of colic

	Controls		
Cases	Seropositive (OD >0.200)	Seronegative (OD ≤0.200)	Total
Seropositive (OD >0.200)	18	2	20
Seronegative (OD ≤0.200)	8	18	26
Total	26	20	46

McNemar's $\chi^2 = 3.60$; p [χ^2] = 0.058; p [exact McNemar's] = 0.109; odds ratio = 0.25 (95% CI = 0.03–1.25 [exact])

	Controls		
Cases	Seropositive (OD >0.600)	Seronegative (OD ≤0.600)	Total
Seropositive (OD >0.600)	5	7	12
Seronegative (OD ≤0.600)	7	27	34
Total	12	34	46

McNemar's $\chi^2 = 0.00$; p [χ^2] = 1.000; p [exact McNemar's] = 1.000; odds ratio = 1.00 (95% CI = 0.30–3.34 [exact])

Table 8
Results of univariable conditional fixed effects logistic regression of variables indicating helminth infection on the risk of colic, in 116 pairs of horses, and in a subset of 46 pairs in which control horses had no known history of colic

Independent (predictor) variable	Nature of variable	Subset of 46 pairs of horses		116 pairs of horses	
		Odds ratio (95% confidence interval)	*p*	Odds ratio (95% confidence interval)	*p*
Anti-*A. perfoliata* ELISA optical density (OD) readings	Continuous	0.051 (−0.92, 1.02) (regression coefficient)	0.919	0.23 (−0.36, 0.81) (regression coefficient)	0.451
Anti-*A. perfoliata* ELISA OD readings[a]	≤0.090	1.00	–	1.00	–
	>0.090 to 0.200	0.54 (0.18, 1.59)	0.260	1.01 (0.47, 2.14)	0.985
	>0.200 to 0.600	0.08 (0.01, 0.64)	0.017	0.38 (0.15, 0.94)	0.036
	>0.600 to 0.800	7.4×10^{-9} (0, ∞)	0.993	0.70 (0.18, 2.77)	0.614
	>0.800	0.33 (0.05, 2.24)	0.255	0.71 (0.25, 2.04)	0.528
Anti-*A. perfoliata* ELISA seropositivity (OD >0.200)[b]	No	1.00	–	1.00	–
	Yes	0.22 (0.05, 1.03)	0.054	0.48 (0.23, 0.98)	0.044
Anti-*A. perfoliata* ELISA seropositivity (OD >0.600)[b]	No	1.00	–	1.00	–
	Yes	1.00 (0.35, 2.85)	1.000	1.28 (0.64, 2.58)	0.481
Presence of fecal *A. perfoliata* eggs[b]	No	1.00	–	1.00	–
	Yes	0.80 (0.21, 3.01)	0.740	0.87 (0.32, 2.42)	0.797
Presence of fecal strongyle eggs[b]	No	1.00	–	1.00	–
	Yes	0.52 (0.22, 1.19)	0.123	0.72 (0.47, 1.08)	0.116
Presence of fecal roundworm eggs[b]	No	1.00	–	1.00	–
	Yes	0.22 (0.03, 1.71)	0.148	0.66 (0.23, 1.92)	0.447
Presence of any fecal helminth eggs[b]	No	1.00	–	1.00	–
	Yes	1.00 (0.32, 3.10)	1.000	0.88 (0.44, 1.77)	0.724

[a] Categorical.
[b] Binomial (dichotomous).

Table 9
Seropositivity to *A. perfoliata* at two levles (OD >0.200 and OD >0.600), as indicated by anti-12/13/kDa IgG(T) ELISA optical density (OD) levels in 116 horses with colic and 116 matched controls

	Controls		
Cases	Seropositive (OD >0.200)	Seronegative (OD ≤0.200)	Total
Seropositive (OD >0.200)	49	11	60
Seronegative (OD ≤0.200)	22	34	56
Total	71	45	116

McNemar's $\chi^2 = 3.67$; p [χ^2] = 0.055; p [exact McNemar's] = 0.080; odds ratio = 0.50 (95% CI = 0.22–1.08 [exact])

	Controls		
Cases	Seropositive (OD >0.600)	Seronegative (OD ≤0.600)	Total
Seropositive (OD >0.600)	17	18	35
Seronegative (OD ≤0.600)	14	67	81
Total	31	85	116

McNemar's $\chi^2 = 0.50$; p [χ^2] = 0.479; p [exact McNemar's] = 0.597; odds ratio = 1.29 (95% CI = 0.60–2.79 [exact])

ELISA OD results were unavailable for one horse with colic and one control horse.

of corresponding unconditional models showed no evidence of poor model fit.

3.6. Association between seropositivity to A. perfoliata and management factors

As the matching of animals in this study was done on the basis of colic status and not on seropositivity, all horses in the dataset with available data were included in the analyses to investigate the association between seropositivity to *A. perfoliata* and management factors. Univariable mixed effects linear regression, with matched pair ID included in models as a random effect to control for any within-pair effect of matching, showed that horses described as being dependent on pasture for a major portion of their diet were significantly more likely to have higher OD levels than non-pasture-dependent animals (regression coefficient = 0.384 [95% CI = 0.055–0.713]; $p = 0.022$). In this model, the random effect term (pair ID) was highly significant (likelihood ratio test [LRT] $\chi^2 = 6.67$; d.f. = 1; $p = 0.005$; intra-class correlation coefficient [ρ] = 0.454). A mixed effects linear model that included both pair ID and veterinarian ID as random effects, to adjust for any additional effect of veterinarian, produced similar results: the regression coefficient for pasture dependence regressed on OD was 0.384 (95% CI = 0.055–0.713; $p = 0.022$). In this model, the random effect term for veterinarian ID was not statistically significant (LRT $\chi^2 = 0.00$; d.f. = 1; $p = 1.000$). Random effects logistic regression with matched pair ID included as a random effect showed that pasture-dependent horses were significantly more likely than non-pasture-dependent horses to have OD levels above 0.66. Such horses were over six times more likely to be seropositive than non-pasture dependent horses, controlling for the effect of matching between pairs (OR = 6.38 [95% CI = 1.20–33.76]; $p = 0.029$). As in the linear regression model, pair ID was highly significant (LRT $\chi^2 = 18.43$; d.f. = 1; $p = 0.001$; $\rho = 0.434$). Furthermore, as for the linear regression model, including veterinarian ID as well as pair ID as random effects in a multilevel model did not greatly alter the results (OR = 6.28 [95% CI = 1.18–33.37]; $p = 0.031$), and veterinarian ID was not significant (LRT $\chi^2 = 0.097$; d.f. = 1; $p = 0.762$). No multivariable random-effects models were constructed, as no other management variables were found to be significantly associated with OD levels or acted as confounders when included with pasture in our analyses, even at the 20% level of significance. Examination of the residuals of the random-effects models showed no evidence of poorly fitting observations.

4. Discussion

This study has provided insight into the occurrence of *A. perfoliata* infection in horses in Southern Ontario, though the criteria for selection of cases and definitions of infection (fecal positive vs. seropositive) require that extrapolation to the larger population be with caution. Fecal microscopy and antibody ELISA testing for *A. perfoliata* infection gave very different results. Tapeworm eggs were detected in the feces of less than 10% of colic and control horses, whereas over 50% of each group showed moderate or high ELISA OD levels. In spite of its lower sensitivity when used in animals with light infections (Proudman and Trees, 1996a,b), the anti 12/13 kDa antibody ELISA used in this study could be a more sensitive method of detecting *A. perfoliata* infection and/or exposure in horses than the Cornell-Wisconsin method used for the detection of fecal parasite eggs. This would not be surprising, as fecal diagnostic methods are considered to have very low sensitivity for detection of *A. perfoliata* infection, especially where only a few tapeworms are present (Slocombe, 1979; Proudman and Edwards, 1992). A predominance of juvenile worms in the infected animals in our study could also have resulted in a high proportion of false negative coprological results. The ability of the Cornell–Wisconsin technique to detect light infections of *A. perfoliata* may be lower than that of the fecal diagnostic method used by Proudman and Edwards (1992), which had a reported sensitivity of 61%. However, there is no evidence that this is the case. The possibility also exists that the antibody ELISA may have detected past as well as current infections. Should this be the case, this would have served to exacerbate the difference between results obtained using the two methods. The extent to which this may have occurred is uncertain; in one study, serum anti 12/13 kDa IgG in four coprologically positive horses declined to below cut-off OD levels within 28 days of anti-cestode treatment (Proudman and Trees, 1996b).

The finding that horses described as being dependent on pasture for a significant part of their diet were over six times more likely to be highly seropositive for *A. perfoliata* (OD >0.600) than non-pasture dependent horses is biologically consistent with an increased risk of exposure to *A. perfoliata* at pasture (Gasser et al., 2005).

No significant association was found between any of the predictor variables investigated (ELISA OD levels and coprological test results) and the risk of colic at the 5% level, with the exception of the (negative) association between seropositivity and colic in some of our conditional logistic regression models (Table 8). An

increased sample size would have provided more power to consistently find statistically significant differences in our subset models. However, it is important to note that, regardless of the model, the measure of association consistently showed a sparing effect between colic and increasing OD levels. The lack of a consistent, significant association, and an odds ratio suggesting a sparing effect between *A. perfoliata* serostatus and colic, are both inconsistent with findings from the UK (Proudman et al., 1998) and the Netherlands (Boswinkel and van Oldruitenborgh-Oosterbaan, 2007), and may in part reflect differences in case definition. In the UK study, cases were diagnosed as spasmodic colic or ileal impaction colic based on quite specific criteria. The latter diagnosis was determined by thorough clinical and surgical assessment in a referral facility, while the diagnosis of ''spasmodic colic'' was determined by the application of exclusion criteria. The term ''spasmodic colic'' refers to sudden-onset abdominal pain of unknown origin which resolves of its own accord or with minimal intervention, and that cannot usually be ascribed to a specific functional or anatomic cause. As in the UK study, cases of colic used in the 2007 work in the Netherlands were also horses referred to an equine teaching clinic for colic of gastrointestinal origin. In contrast, cases in our work were horses diagnosed with any type of colic by veterinary practitioners in the field, and, for the most part, there was no opportunity to achieve a definitive diagnosis, or to rule in or out specific causes of colic. In our study, therefore, many of the cases may not have met the diagnostic criteria used in the two European studies.

Despite efforts to match cases and controls in the work carried out by Proudman et al. (1998), only 48% of controls were from the same premises as the corresponding cases, potentially influencing findings in favour of identifying a difference. This is discussed further in the next paragraph. On the other hand, in our study, the close association observed between serological status and grazing emphasizes the possible significance of management factors and the need to consider them when identifying controls, but does not negate the significant role proposed for tapeworms in colic.

Our decision to match by stable was highly influenced by the design of the case-control study conducted by Proudman et al. (1998). However, as indicated, in the UK study, not all cases were successfully matched to a control from the same stable, whereas nearly all cases were successfully matched in our study. The work carried out by Boswinkel and van Oldruitenborgh-Oosterbaan (2007) made no attempt to match horses by stable; controls were randomly selected from equine farms throughout the Netherlands. In our work, as in the UK study, matching of horses by age was considered prudent because of the reported dependence of serological response of horses on age (Proudman et al., 1997). However, the differences in the degree to which horses were matched by stable may account for differences between our findings and the UK and Netherlands studies: our data revealed a strong correlation between ELISA OD among animals from the same stable. As a consequence, matching by stable may have forced the exposures between cases and controls to be more similar than would be expected in the source population. It should be noted that matching in case-control studies does not remove the effect of a confounding variable, but allows for a more efficient stratified analysis if the variable is rare and is a true confounder. Matching on non-confounders can lead to over-matching and a loss of analytical efficiency (Rothman and Greenland, 1998; Dohoo et al., 2003). Future case-control studies estimating the association between colic and seropositivity to *A. perfoliata* may benefit from using analytical methods alone to control for potential confounding variables, rather than matching. The commonality of exposure of case-control pairs to *A. perfoliata* revealed in our study that accounting for herd effects is essential in the design of future studies. For instance, understanding the relationship between pasture grazing and colic may be more appropriately addressed through cross-sectional studies that assess individual level risk for colic while controlling for clustering at the herd/stable level. Our findings have certainly found a relationship between exposure to pasture and OD levels, but the matched design appears to have limited our ability to detect any existing relationship between pasture and colic; previous research has shown that horses with access to pasture may have a reduced risk of colic (Cohen et al., 1999; Hudson et al., 2001).

5. Conclusion

In summary, our work showed no evidence of a statistically significant association between seropositivity to *A. perfoliata* and risk of colic, as defined here, in Ontario horses. Because of differences in case definition, our results do not disprove the findings of Proudman et al. (1998), but do question the role played by tapeworm infections in self-limiting cases of equine colic that are treated on a routine basis by Ontario practitioners.

Acknowledgements

The authors acknowledge the contributions of the veterinary practitioners and horse owners who provided

the data used in this study. Dr. Noah Cohen, Texas A&M University, is thanked for kindly providing copies of colic surveys that served as the template for the questionnaire used in this work. Serological analyses and interpretation of data were conducted by Diagnosteq Laboratories, University of Liverpool, United Kingdom. Financial support for this project was provided by Pfizer Animal Health Canada, Pfizer Animal Health USA, the E.P. Taylor Foundation, and the Ontario Ministry of Agriculture, Food and Rural Affairs. The animal utilization protocol used in this study was approved by the Animal Care Committee, University of Guelph.

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