Veterinary Parasitology 197 (2013) 580–585

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

The association between *Anoplocephala perfoliata* and colic in Swedish horses—A case control study

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ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form 11 July 2013
Accepted 16 July 2013

Keywords:
Equine
Colic
Tapeworm
Anoplocephala perfoliata
ELISA
Fecal sample

ABSTRACT

A case-control study was performed to investigate the association between colic of all types in Swedish horses and infection with the equine tapeworm *Anoplocephala perfoliata*. Colic cases were defined by clinical signs consistent with the presence of abdominal pain, and the control horses had no signs of colic within the last year but attended a clinic for other reasons. Blood and fecal samples were collected by veterinarian from 67 horses with signs of colic and 67 control horses. The sera were analyzed using serodiagnostic assay anti-12/13 kDa IgG(T) ELISA. The fecal samples, 30 g from each horse, were analyzed with a modified sugar salt flotation method with a density of 1.280. A significant association was found between the presence of *A. perfoliata* eggs in feces and colic with a 16 times higher risk of colic if eggs had been observed in fecal samples. However, there was no significant association between colic and the median OD-values in the serological diagnosis, nor when recommended cut-offs were used. The study concludes that *A. perfoliata* is a risk factor for colic in Swedish horses and it suggests that the modified flotation method can be used as a diagnostic tool for identifying horses at risk.

1. Introduction

Colic is a common, medical symptom in the equine population (Scantlebury et al., 2011) and the abdominal pain often has a multi-factorial origin (Reeves, 1997). Medical colic is a frequent reason for veterinary attendance in the equine practice (Traub-Dargatz et al., 1991), and according to horse owners colic is one of the major health concerns in horses (Mellor et al., 2001). *Anoplocephala perfoliata* was previously regarded as a relatively harmless endoparasite of little or no importance (Soulsby and Mönnig, 1968), but in the 1980s several reports of a possible association between tapeworm infection and gastrointestinal disease were presented (Barclay et al., 1982; Beroza et al., 1983; Owen et al., 1989).

There have been few studies of the possible association between colic and infection with *A. perfoliata* and the results reported are not consistent. In a case-control study performed in the UK, a significant association between *A. perfoliata* and spasmodic colic was seen using serological (ELISA) as well as coprological methods (Proudman et al., 1998). However, a Canadian case-control study, based on the same methods, did not reveal any association between colic and *A. perfoliata* infection (Trotz-Williams et al., 2008). Histopathologically, *A. perfoliata* can cause inflammation of the lamina propria and mucosal damage at the region of the ileocecal junction, and there is an association between the number of worms present and the severity of the histological changes (Kjaer et al., 2007; Nilsson et al., 1995; Pavone et al., 2011; Pearson et al., 1993). Furthermore, studies have observed significant gross thickening and fibrosis of the ileocecal junction and several changes in neuronal cells in horses where more than 20 tapeworms were present (Fogarty et al., 1994; Pavone et al., 2011). Such pathological findings could possibly be linked to intestinal motility

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0304-4017/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.vetpar.2013.07.020

disorders and explain the observed association between infection of *A. perfoliata* and spasmodic colic (Proudman et al., 1998).

A. perfoliata is the most common equine tapeworm, and the predilection site is the cecal side of the ileocecal junction (Dunn, 1978). The worldwide reported prevalence is between 18 and 82% (Bain and Kelly, 1977; Lyons et al., 1983; Mfitilodze and Hutchinson, 1989; Pearson et al., 1993; Reinemeyer et al., 1984). In Sweden, a prevalence of 65% has been reported (Nilsson et al., 1995).

Today there is no 'gold standard' for detecting a tapeworm infection in horses but there are several methods available. Fecal analyses have limitations for detecting tape worm eggs, but with higher burdens of tapeworms the sensitivity of the coprological methods increases (Proudman and Edwards, 1992; Williamson et al., 1998). The McMaster flotation and other unmodified flotation techniques based on <5 g of feces have been shown to have very low sensitivity for the tapeworm, whereas a modified flotation technique method, where 10 times more material is used, has been described with a sensitivity of 92% if the horse is harboring 20 tapeworms or more (Beroza et al., 1987; Meana et al., 1998; Nilsson et al., 1995; Proudman and Edwards, 1992). Two different ELISA techniques have been developed for detection of antibodies in serum where the specific IgG(T) is the target for the method (Hoglund et al., 1995; Proudman and Trees, 1996a,b). However, the optimal cut-off level for the commercial ELISA seems to vary between countries (Kjaer et al., 2007; Proudman and Trees, 1996b) which makes the results somewhat difficult to interpret. Moreover, the knowledge is scarce about how long persisting levels of antibody titers will last after worms no longer are present, although some studies have been performed (Barrett et al., 2005; Proudman and Trees, 1996a). Persisting antibodies can make it difficult to distinguish false positives from true positives (Kjaer et al., 2007).

Investigating how *A. perfoliata* affects horses in different areas is of importance because the climate and management traditions vary between countries and regions. As the possible association between colic and *A. perfoliata* infection has not been investigated in Sweden, and the available diagnostic techniques have not been evaluated, the aim of this case-control study was to investigate the association between *A. perfoliata* infection and signs of colic in Swedish horses using two diagnostic techniques: a modified flotation technique for fecal samples and a serological ELISA method.

2. Material and methods

2.1. Study design

This study was designed as a prospective case-control study, and one referral clinic and one private equine practitioner in the south of Sweden participated in the study. A horse with signs of colic, i.e. clinical signs consistent with the presence of abdominal pain that needed treatment from a veterinarian, was classified as a case horse. For each horse with signs of colic a control horse in the same clinic and of about the same age was chosen. The control horse should have arrived at the clinic within 1 week after the colic. Moreover, control horses should not have had colic within the previous year. The study period was from January 2010 to the end of November 2012. Fecal and blood samples were collected by veterinarians from 67 colic horses and 67 control horses and sent by post to arrive the next day to the National Veterinary Institute (SVA). By using a questionnaire, the owners and veterinarians were asked to provide information about age and breed of the horse, previous signs of colic, and history of anthelmintic treatments within the last year.

2.2. Processing of samples

Fecal samples were examined for tapeworm eggs using the modified flotation technique described by Beroza et al. (1987). Briefly, 30 g of feces was mixed with 60 ml tap water, dispersed by stirring and sieved with a strainer (aperture 100 mesh). The sieved water was then distributed into four 15 ml test tubes and centrifuged $1000 \times g$ for 10 min. Then the supernatant was removed by aspiration, the respective pellets were shaken with Vortex and then the tubes were filled up with a sugar salt solution to form a convex meniscus. The sugar salt solution is a saturated sodium chloride solution with 50% glucose with a density/specific weight of 1.280 (360 g of natrium chloride was added to 1000 ml of tap water and boiled into a clear solution, and then 500 g of glucose was added). Cover glasses (18 mm × 18 mm) were put on the top of the tubes before centrifugation (for 5 min at $214 \times g$, no brakes) in a swing out centrifuge with specific carriers that can hold cover glasses in position during centrifugation. After 5 min all four cover glasses were transferred to microscope slides and examined under a microscope at 40–100× magnification. Sera were separated from the blood samples and stored in −20 °C before shipment in dry ice to the Diagnosteq Laboratory (Liverpool, UK) for analysis with the *A. perfoliata* antibody ELISA developed by Proudman and Trees (1996a). According to the Diagnosteq Laboratory, there are three recommended cut-off values for the ELISA: <0.200 indicates zero/low infection, 0.201–0.700 is classified as moderate infection intensity and >0.700 is considered as high infection intensity.

2.3. Statistical analysis

Descriptive statistics were used to present the results of the distribution of the dependent variable (being a horse with colic or not) and independent variables (age, gender, breed, presence of *A. perfoliata* eggs and OD values of the ELISA). Associations between the dependent variable and the independent variables were investigated using *t*-test, Fishers's exact-test, Wilcoxon rank-sum test, Chi^2 test and univariate logistic regression models as appropriate. Continuous variables not linearly related to the outcome were categorized using the quartiles as cut-points, or if available by using biologically important or recommended cut-points (e.g. by the analyzing laboratory). A *P*-value of <0.05 was considered statistically significant. All statistical