Biological Forum – An International Journal **7**(1): 106-110(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Survey on Fecal Gastrointestinal Parasitic Helminthes in Horses of Jokey clubs in Ardabil city, Iran

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(Received 14 December, 2014, Accepted 15 January, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: This study aimed to estimate infection prevalence of strongyle, *Oxyurisequi* and *Parascaris equorum* species and intensity of infection with strongyles in horses of jokey clubs in Ardabil city, Iranand investigate associations between infection and horse age, sex and owner-reported use of anthelmintics. In a cross-sectional survey, fresh fecal samples were obtained from 50 randomly selected horses and worm egg counts performed using a validated field laboratory kit. Details of anthelmintic use were collected using a standardised face-to-face owner questionnaire. Infection prevalence estimates for each species were calculated, as were infection intensity estimates for strongyle species. Logistic regression was used to investigate associations between exposure variables and infection status/intensity. Prevalence of strongyle infection was 34% (17:50) in this survey. Cylicostephanuslongibur satus, Cylicostephanusgoldi and Cylicocyclusnassatus were founded species of gastrointestinal parasitic helminthes in this study. Decreasing strongyle infection intensity was associated with use of proprietary equine anthelmintic products. *Oxyuris equi* and *Parascaris equorum* species was not found in this study.

In conclusion, strongyle infection is endemic in horses of jokey clubs in Ardabil city, Iran but proprietary equine anthelmintics assist in managing infection. The apparent lack of age-acquired immunity to *P. equorum* infection may deserve further investigation. Although *O. equiinfection* is less widespread, measures to protect younger animals may be appropriate.

Key words: Gastrointestinal, parasitic helminthes, horses, Ardabil city, Iran.

INTRODUCTION

There are an estimated 110 million equines (horses, donkeys, mules) in the developing world (FAOSTAT, 2008) where they provide an essential, widely used resource for traction and under saddle as a means of transport due to economic and/or topographical constraints on motorised alternatives (Ramaswamy, 1994; Gebreab, 1998; Pritchard *et al.,* 2005). A large study looking at the association between poverty and animal disease (Perry *et al.,* 2002) identified gastrointestinal (GI) parasitism as one of the most important problems for equids in developing countries. Studies conducted in Ethiopia and Mexico estimate the prevalence of endoparasite infections at over 90% in horses (Valdez-Cruz *et al.,* 2006; Fikru *et al.,* 2005; Gebreab, 1998) and over 80% in donkeys (Burden *et al.,* 2010; Getachew *et al.,* 2010; du Toit *et al.,* 2008). Gastrointestinal parasite burdens also seem to be substantial in donkeys in The Gambia (Mattioli *et al.,* 1994) and Republic of South Africa (RSA) (Matthee *et al.,* 2000; Wells *et al.,* 1998).

The aims of this study were to quantify the GI parasite burden of horses of jokey clubs in Ardabil city, Iran and to identify factors that may be associated with presence and/or severity of helminth infection. Specific objectives were to: (i) estimate the prevalence of strongyle, *P. equorum* and *O. equiinfection* and severity of strongyle infection in horses of jokey clubs in Ardabil city, Iran; (ii) investigate the association between intensity of strongyle infection and horse age, horse sex and owner-reported use of proprietary anthelmintic products.

MATERIALS AND METHODS

A. Study design, period and area

This study formed part of a cross-sectional baseline survey of health and welfare in horses of jokey clubs in Ardabil city, Iran, undertaken between April and June 2014 in 5 jokey clubs in Ardabil city, Iran . The survey was the first stage of an impact assessment study that aimed to investigate changes in horse welfare following implementation of intervention programmes.

B. Sample size determination

Sample size calculations were performed in the context of the main study objectives. Calculations for a cluster randomised study (assuming a significance level of 5%, 80% study power and intra-class correlation coefficient of 1-5%) indicated that 50 horses/owners would need to be sampled/questioned.

C. Data collection

The research team visited each selected jokey clubs in Ardabil city, Iran on a single occasion. Each owner was interviewed in local language using a pre-tested, purpose-designed questionnaire. Questions included what product(s) were used for purposes of preventing/treating the horse for endoparasites. Each horse was examined according to a standardised comprehensive pre-tested protocol by a veterinary-trained member of the research team; findings were recorded in a standardised format. Clinical examination variables that were recorded included age (as estimated by dental examination) and sex.

D. Collection of samples

With the owner's permission, a fresh faecal sample for faecal egg count (FEC) was collected from each horse examined, either per rectum or, if this was not possible, via identification of a freshly voided sample. Each faecal sample was individually stored in an electrically powered cool box for transfer to purpose-made temporary laboratory facilities at the team base location and kept cool prior to processing.

E. Processing of samples

Samples were processed within 48 hours of their arrival at the temporary laboratory using the FECPAK (FecPakInternational) worm egg counting system which, like the McMaster technique, is based on the flotation-dilution principle (Presland *et al.*, 2005). A sample of at least 20g was mixed thoroughly in a plastic bag with three times the volume of water. Once fully mixed, 45ml of the mixed suspension was removed, made up to 230ml with saturated salt solution and then poured through a 1mm aperture filter to remove coarse debris. An aliquot was then removed from the filtrate and place in a custom-made acrylic counting chamber using a volume of 1ml under the pre-marked grids. The slide was left for 30-60 seconds to allow time for nematode eggs to float to the top of the chamber and then the number of eggs was counted under a microscope. Based on visual morphological identification (Soulsby, 1982), each egg counted was classified as strongyle, *P. equorumor O. equi.* The number of eggs per gram (epg) was calculated by multiplying the number of eggs counted by a factor of 25 to account for dilution applied during processing.

RESULTS

A. Descriptive results

Faecal samples were collected from 50 horses, 50% of which were male. Age based on dentition was recorded for all horses. Horse age was normally distributed, with a mean age of 8.3 years (standard deviation 4.99, range 2-24 years). The coprological prevalence of strongyle infection was 34% (17:50). Strongyleepg values were non-normally distributed with a median of 752 (interquartile range 227-984). Table 1 shows the prevalence of infection intensity categories for the 50 horses sampled.

For purposes of this analysis, responses were categorised as recognised equine anthelmintics or 'other'. Proprietary equine anthelmintics included Equest (Moxidectin, Fort Dodge Animal Health), Equimax (Ivermectin/Praziquantel, Pfizer Animal Health), Eqvalan (Ivermectin, Merial Ltd), Noromectin (Ivermectin, Norbrook Laboratories) and Panacur (Fenbendazole, Schering Plough Animal Health).

Table 1: The prevalence of infection intensity categories for the 50 horses fecal sampled.

Parasitic helminth	Number of infected horse	% prevalence
Cylicocyclus nassatus	11	22
Cylicostephanus longibursatus	4	8
Cylicostephanus goldi	2	4

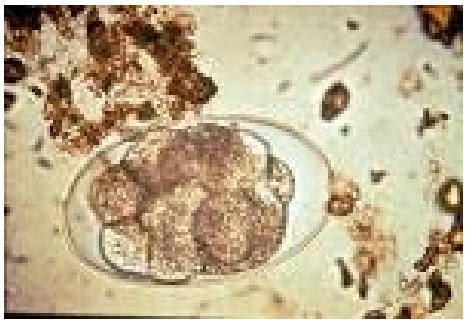

Fig. 1. Egg of horse strongyle in fecal smear.

B. Associations between independent variables and intensity of strongyle infection

The crude OR represents the relative odds of a horse being in a higher intensity category for a one unit increase in the independent variable. For example, the odds of a three-year-old horse being in a non-zero epg category as compared to a two-year-old horse are 0.94. Increasing age and use of proprietary equine anthelmintics were significantly associated with decreased odds of higher intensity strongyle infection. However, when adjusted for the use of a proprietary equine anthelmintic, the ORs were 0.96 for age ($p = 0.07$) and 1.24 ($p = 0.38$) for sex. Results of multivariable analysis indicated that only use of a proprietary equine anthelmintic was associated with significantly reduced odds of being in a higher intensity infection category.

DISCUSSION

This is the first study to report equine helminth infection prevalence and intensity of infection in horses of jokey clubs in Ardabil city, Iran. The estimate for strongyle prevalence (34%) is not similar to that of recent studies in Ethiopia (Fikru *et al.,* 2005) and Mexico (Valdez-Cruz *et al.,* 2006), both estimating prevalence in horses to be 91%. Studies in donkeys report FEC prevalence estimates of 95-100% (Wells *et al.,* 1998) and 72% (Matthee *et al.,* 2000) in RSA; 83% in The Gambia (Mattioli *et al.,* 1994); 99% in Ethiopia (Getachew *et al.,* 2010); and similar estimates of 80% (Burden *et al.,* 2010) and 81% (du Toit *et al.,* 2008) in Mexico. Potential variation arising from species differences and differences with respect to topography, climate, animal working conditions and seasonality make direct comparisons difficult. The differences in intensity of infection could be due to variations in parasite biology relating to climatic conditions, pasture infection intensity relating to grazing practices and/or differences in use of anthelmintics; detailed information on such variables would be required to determine their importance.

This study was conducted during a single seasonal period from late autumn into winter, when night time temperatures were cold and there was little rainfall. Previous studies in several African countries (Ayele, 2006; Fikru *et al.,* 2005; Yoseph *et al.,* 2005; *Mushi et al.,* 2003) indicate that such conditions tend to be associated with lower prevalence and intensity of helminth infection, so the estimates reported here are likely to be higher in the warm and humid summer season. A study by Getachew *et al.,* (2008b) which looked at FEC in horses over a period of two years in Ethiopia confirmed that counts were highest in the long rainy season as compared to the dry season. There seemed to be an inverse association between age and intensity of strongyle infection, i.e. as horses get older the odds of higher egg count are reduced. However, this association became only marginally significant when adjusted for use of anthelmintics, which seems to suggest that use of anthelmintics varies between age groups. Since only horses of jokey clubs in Ardabil city, Iran were included in this study the minimum age of horses was two years old; most previous studies investigating the association between endoparasitism and age have included foals and yearlings, making direct comparisons of age associations difficult. Purpose-designed studies including key age groups 175 for each type of equine endoparasite species would be required to investigate age associations further and determine how they compare with those reported elsewhere.

This study identified a trend towards significance for increased odds of greater strongyle infection for female horses, but this association became less strong and non-significant when adjusted for use of proprietary anthelmintics in multivariable analysis. As for age, this seems to indicate that use of anthelmintics differs between male and female horses. This finding supports anecdotal information derived from discussions with owners during the survey,

suggesting that female horses are managed differently from male horses. However, the reasons why and nature and extent to which husbandry practices vary between age and sex groups are unclear and require further investigation; such studies could identify specific needs for owner education that could reduce endoparasite burdens and also improve horse welfare in general. Although the cross-sectional study design meant detailed investigation of worming programmes was not possible, the significant inverse association between reported use of a recognized equine anthelmintic product and strongyle epg category appears to indicate that, when used, such products are achieving the desired effect.

The cost of proprietary products relative to local incomes (World Bank, 2008) means economic constraints are considerable although a previous study highlighted the importance of other types of constraint including accessibility of services (Heffernan, 2001) so any proposed worm management programmemust be designed to be financially viable for local owners. Clinical improvements associated with administration ofproprietary anthelmintics should also be investigated further to ensure that such programmes result in real benefits for horses and owners (Crane *et al*., 2008).

CONCLUSION

In conclusion, strongyle infection is endemic in horses of jokey clubs in Ardabil city, Iran but proprietary equine anthelmintics assist in managing infection. However, access to such products is limited due to economic constraints which must be taken into account when designing viable worming programmes.

ACKNOWLEDGMENTS

The authors would like to thanks Tabriz Branch, Islamic Azad University for the laboratory Instruments supports of this research and Mr. Mohammad rezakazemi for help in collection of fecal samples.

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