Indian J. Anim. Hlth. (2017), 56(2):257-264

Short Communication

ASSOCIATION OF RISK FACTORS WITH THE PREVALENCE OF GASTROINTESTINAL PARASITES OF DONKEYS *(EQUUS ASINUS)* FROM SOUTHERN PUNJAB

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The coproscopic examination of 80 faecal samples of donkeys collected from southern Punjab districts revealed an overall prevalence rate of 41.25% (33/80) for gastrointestinal parasites. The prevalence rates for strongyle, *Parascaris equorum*, mixed parasitic infections (*Strongyloides westeri* and strongyle) and amphistomes were 31.25, 6.25, 2.5 and 1.25%, respectively. Furthermore, quantitative analysis of positive faecal samples revealed mild to severe type of intensity for strongyle infection as indicated by eggs per gram ranging from 200-2200 (742±86.4). Regarding *P. equorum* infection, the eggs per gram value ranged from 150 to 250 (200±20.4) indicating mild type of infection. Coproculture studies revealed small strongyles (Cyathostomes) as predominant species (58.80%) of which 46.0% belonged to type A and 38.0% to type C, respectively. Amongst the large strongyles, highest proportion was recorded for *Strongylus edentatus* (17.6%) followed by *S. vulgaris* (15.4%) and least for *S. equinus* (8.2%). Age and sex were not associated with the prevalence of parasitic infections, while season, districts and farm types were significantly associated with the risk of gastrointestinal parasites (P<0.01).

Key words: Donkeys, Gastrointestinal parasites, Prevalence, Risk factors, Southern Punjab

Donkeys (*Equus asinus*) are considered to be among the early domesticated equines and have been used as "beast of burden" for thousands of years (Saul *et al*., 1997).

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The total estimated population of donkeys in India and Punjab is 0.32 and 0.003 million, respectively (Anonymous, 2012). In India, donkeys are considered to be the cheapest and easiest means of transport, suiting needs of washer man, potters, house builders, brick manufactures and Vanjara community engaged in earth work till today (Parsani *et al*., 2013).

Despite multi-utilities and much impact on living and earning of poor communities, the donkeys are often under-appreciated, overburdened, under-fed and left malnourished, reared in poor managemental conditions, and not taken care of in ailments (Jajere *et al*., 2016). Among the parasitic diseases, donkeys suffer from a variety of gastrointestinal (GI) parasites of which strongyle infections are the most common. The strongyles are active bloodsuckers and in huge numbers cause severe anaemia, weakness, emaciation, colic and diarrhoea (Burden *et al*., 2010). Although information is available regarding the prevalence of GI parasites in donkeys from India, there appears to be no published report on this aspect from Punjab state. Hence, present study was undertaken to determine the prevalence of GI parasites affecting donkeys along with assessment of associated risk factors in districts of southern Punjab.

A total of 80 freshly laid faecal samples from donkeys of seven districts (Barnala, Bathinda, Faridkot, Fazilka, Sri Muktsar Sahib, Mansa and Sangrur) of southern Punjab were randomly collected during one year study period (January 2016 to December 2016). After collection, faecal sample was properly labelled, kept in an icebox and transported to the Postgraduate Laboratory, Department of Veterinary Parasitology, College of Veterinary Sciences, GADVASU, Ludhiana for qualitative and quantitative examination of GI parasitic infection(s). Other relevant information regarding age, sex, farm management system, district and time of collection was also recorded at the time of sampling on a separate proforma.

The faecal samples were first subjected to standard qualitative coproscopic examination using direct smear, sedimentation and floatation techniques for screening of parasitic eggs/oocysts by microscopic examination (Soulsby, 1982). For quantitative faecal sample examination, standard McMaster's technique was used to calculate the eggs per gram (EPG) of faeces and the intensity of infection was categorized (Soulsby, 1982). The coproculture examination was also performed on representative number of

samples positive for strongyles by transferring the faecal, charcoal and sterilized soil mixture to a Petri plate that was kept in an incubator at 26-27°C for 6-7 days. Finally the third stage larvae (L_3) were collected by Baermann's technique and subjected to morphological examination for identification of the genera. The hatched out larvae of large strongyles were identified as per the keys of Manual of Veterinary Parasitological Laboratory Techniques, Ministry of Agriculture, Fisheries and Food (Anonymous 1986) and for small strongyles (Cyathostomes) the identification was made as per Kornas *et al*. (2009) wherein the characteristics like larval size (total length, body length, oesophagus length, intestinal cells length and gap between cells and start of sheath); presence/absence of sheath; number and shape of intestinal cells; arrangement of intestinal cells and clarity of the intestinal cells were taken into consideration. All data analyses were performed by using statistical software program (SAS software Version 9.3, CARY, USA). Association between the prevalence of GI parasitic infection and various risk factors was carried out by Chi square (χ^2) test.

An overall prevalence of 41.25% was recorded for GI parasites in donkeys of southern Punjab (Table 1). In terms of parasitic composition, the prevalence rates indicated strongyle (31.25%) infection to be the maximum and amphistome (1.25%) as minimum. The quantitative analysis of faecal samples positive for strongyle revealed eggs per gram (EPG) of faeces ranging from 200 to 2200 (742±86.4) thereby showing mild to severe type of intensity (Soulsby, 1982) whereas for *P. equorum* infections, the value ranged from 150 to 250 (200±20.4) indicating mild type of infection (Soulsby, 1982).

Amongst the strongyles, coproculture studies revealed highest proportion of small strongyles larvae (Cyathostomes) as 58.8%, of which 46.0% were identified as type A, 38.0% as type C while 16.0% could not be identified. The identification of types of cyathostomes was done as per keys given by Kornas *et al*. (2009). Regarding, the large strongyles (41.2%), *Strongylus edentatus* was the predominant species (17.6%), followed by *S. vulgaris* (15.4%) and *S. equinus* (8.2%). The details of larvae in terms of micrometrical observations are presented in Table 2 and were carried out for both small and large strongyles (MAFF, 1986).

Table 1. Prevalence and risk factors associated with the occurrence of GI parasites in donkeys of southern Punjab districts

Risk factors	Number examined	Number positive	Strongyle (%)	*Parascaris equorum* (%)	Amphistome (%)	Mixed# (%)	Overall prevalence (%)
Season							
Rainy	49	20	12 (24.4)	05 (10.2)	01 (2.04)	02 (4.08)	40.8
Summer	20	12	12 (60.0)	00 (0.0)	00 (0.0)	00 (0.0)	60.0
Winter	11	01	01 (9.09)	00 (0.0)	00 (0.0)	00 (0.0)	9.09
χ^2 value							15.9310**
Sex							
Male	26	10	08 (30.7)	01 (3.85)	00 (0.0)	01 (3.85)	38.5
Female	54	23	17 (31.48)	04 (7.40)	01 (1.85)	01 (1.85)	42.5
χ^2value							1.1706
Age							
<2 year	08	02	01 (12.5)	01 (12.5)	00 (0.0)	00 (0.0)	25.0
2-4 year	28	11	08 (28.5)	01 (3.57)	01 (3.57)	01 (3.57)	39.2
>4 year	44	20	16 (36.3)	03 (6.81)	00 (0.0)	01 (2.27)	45.4
χ^2 value							8.8967
Districts							
Barnala	04	01	00 (0.0)	01 (25.0)	00 (0.0)	00 (0.0)	25.0
Bathinda	18	10	08 (44.4)	02 (11.1)	00 (0.0)	00 (0.0)	55.5
Faridkot	13	04	02 (15.3)	01 (7.7)	00 (0.0)	01 (7.7)	31.0
Fazilka	11	03	02 (18.1)	00 (0.0)	00 (0.0)	01 (9.09)	27.2
Sri Muktsar Sahib	11	00	00 (0.0)	00 (0.0)	00 (0.0)	00 (0.0)	0.0
Mansa	14	11	11 (78.5)	00 (0.0)	00 (0.0)	00 (0.0)	78.5
Sangrur	09	04	02 (22.2)	01 (11.1)	01 (11.1)	00 (0.0)	44.4
χ^2value							43.7081**
Farm types							
Organised	00	00	00 (0.0)	00 (0.0)	00 (0.0)	00 (0.0)	0.0
Unorganised	80	33	25 (31.25)	05 (6.25)	01 (1.25)	02 (2.5)	41.25**
Total	**80**	**33**					**41.25**

Mixed infection: *Strongyloides westeri* + Strongyle spp.
** P<0.01

Table 2. Micrometrical observations of larvae of strongyles in donkeys in southern Punjab districts

Species of strongyles	Mean values (X± SE)				
	A(µ)	B(µ)	B1(µ)	B2(µ)	B3(µ)
Cyathostomes	723±4.80	420±1.56	139±0.27	220±0.39	61±0.24
Strongylus vulgaris	927±4.22	738±2.80	180±0.73	480±0.71	78±0.46
Strongylus equinus	858±4.59	638±1.84	154±0.45	401±0.73	83±0.57
Strongylus edentatus	748±4.70	500±3.66	170±0.49	280±0.62	50±0.36

A=total length, B=body, B1=oesophagus, B2=intestinal cells, B3=gap between cells and start of sheath, X=mean, SE=standard error

Amongst various risk factors, season was found as a major factor for variation in the prevalence of the GI parasites with highest prevalence recorded in summer and least in winter and the data was statistically significant (P<0.01) (Table 1). Regarding the age-wise prevalence, higher rate was observed in old animals (>4year) and least in young (<2year) age groups, but the data was statistically non-significant (Table 1). Regarding the sex-wise prevalence, females showed comparatively higher prevalence rates as compared to males but the variation was statistically non-significant (Table 1). The districts were also found to be a major risk factor in the prevalence of the GI parasites and the data was statistically significant (P<0.01) with highest prevalence recorded in Mansa and least in Sri Muktsar Sahib (Table 1). Farm types were also found to be a predominant risk factor in the prevalence of the GI parasites and the data was statistically significant (P<0.01) with higher prevalence rates observed in unorganised farms (Table 1).

The results obtained in this study are in close agreement to those obtained from Sudan and Ethiopia (Sawsan *et al*., 2008 and Mangassa and Mhatebu, 2016). However, workers from different countries worldwide including India, have reported relatively higher prevalence rates ranging from 55.7%-98.3% (Parsani *et al*., 2013 and Jajere *et al*., 2016). Furthermore though the strongyle infection was reported to be highest (31.25%) in the present study, the percent prevalence was comparatively lower than that reported by many workers from various regions of India. Also the prevalence of *P. equorum* observed in this study was lower than that reported by many workers worldwide. These differences might be attributed to various factors such as variations in sample sizes, sampling periods, geographical location, age groups, deworming strategy, nutritional status,

managemental and environmental factors, accessibility to veterinary clinics and methods/criteria employed for sample collection and analysis (Parsani *et al*., 2013 and Jajere *et al*., 2016).

Lower prevalence rate of equine amphistomosis reported in the present study might be attributed to the fact that the current investigation was carried out in southern Punjab districts where the geo-climatic conditions are hot and dry with loamy-sandy soil, which are not favourable for survival and perpetuation of snails, thereby, resulting in lower prevalence of amphistomes. The EPG value observed in this study is comparative to those obtained by Adam *et al*. (2013) and Sheferaw and Alemu (2015) from Sudan and Ethiopia who reported the range of EPG for GI parasites as 100-2900 (642.2±38.8) and 100-1600 (726.9±22.0), respectively. Report of cyathostomes to be predominant amongst the Strongyle spp. is in line with those of various workers that might be due to the use of broad spectrum anthelmintics like benzimidazoles and macrocyclic lactones which result in drastic reduction in worm populations particularly large strongyles, thereby, leading to higher infection rates of small strongyles in equines (Konigova *et al*., 2002).

As far as seasonal prevalence is concerned, the results of the current study are in close proximity with the observations of Parsani *et al*. (2013) who reported the prevalence rates of GI infections to be maximum in summer and minimum in monsoon. Presumably, the variations in humidity and temperature directly influence the hatching of parasitic eggs and growth rates of intermediate hosts (if any) resulting in variable prevalence rates. Regarding the age-wise prevalence, the strongyle infection was found higher in old animal which is congruent to findings of Takele and Nibret (2013) from Ethiopia. The probable reason for this may be differences in number of samples in the respective category. Further, the pre-patent period of small strongyles is very short than large strongyles (Soulsby, 1982) and this variation may cause their lower prevalence rates in younger donkeys. Higher percent prevalence of ascarid infection in younger animals could be attributed to the development of acquired immunity with increasing age (Soulsby, 1982).

Regarding the sex-wise prevalence, findings similar to the current study have been reported by various workers worldwide who observed the prevalence rate of all GI parasites to be non-significant and highest in females as compared to males (Tesfu *et al*., 2014 and Jajere *et al*., 2016). As regards farm types, the results of the current study are in close proximity with the observations of Jajere *et al*. (2016) who reported statistically higher prevalence of helminthic infections in donkeys from Nigeria having with poor (thin) body condition, rural settlements, not dewormed and raised under poor management systems ($P<0.001$).

ACKNOWLEDGEMENT

The authors are grateful to the Dean, Postgraduate Studies, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab for providing the necessary facilities. Thanks are also due to the Veterinary Officers for help rendered in collection of samples for the smooth conduct of study.

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Article received on 05.06.2017 and accepted for publication on 12.08.2017