Research Article

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PREVALENCE OF GASTROINTESTINAL PARASITES IN HORSES OF SOUTHERN PUNJAB DISTRICTS

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ABSTRACT

A total of 257 fresh faecal samples of horses randomly collected from seven districts of southern Punjab were examined microscopically for presence of gastrointestinal (GI) parasites. The coproscopic examination revealed an overall prevalence of 19.06 % for GI parasites. Among the various GI parasites recorded, the prevalence of strongyle sp., *Parascaris equorum*, mixed infection (*Strongyloides westeri* and strongyle sp.), amphistomes, *Eimeria* sp. and *Trichuris* sp. were 12.06%, 2.72%, 1.56%, 1.56%, 0.78% and 0.38%, respectively. Furthermore, quantitative analysis of the positive faecal samples revealed mild to moderate intensity of infection from 50 to 1500 (441.93±61.5) and 100 to 200 (142.85± 17.0) for strongyles and *P. equorum*, respectively. Coproculture studies for strongyles revealed small strongyles (Cyathostomes) as predominant species (65.20) which consisted of 48.2% type A and 41.4% type C larvae. Amongst the large strongyles, highest proportion was recorded for *Strongylus edentatus* (15.70%) followed by *S. vulgaris* (10.40%) and *S. equinus* (8.70%). Seasons and farm types were significantly associated with the prevalence of GI parasitic infections. The results of present investigation revealed strongyle sp. to be the major parasitic infection among the horses in southern Punjab.

Key words: Horses, Gastrointestinal Parasites, Prevalence, Southern Punjab, Strongyles

Horses play a significant role in the human civilizations worldwide as they have been utilized by the people for sports, warfare, carrying heavy loads and agricultural operations. As regards Punjab state, the equine population stands as 32,860 horses and ponies (Anonymous, 2012). The horses suffer from a wide spectrum of disease causing pathogens of which gastrointestinal (GI) helminths are very common and present a serious challenge to health and welfare issues of the equids worldwide. The GI parasites caused economic losses through reducing working capacity, reduction in food intake and lower weight gains, colic, diarrhoea, treatment cost and mortality in heavy parasitized animals (Buzatu *et al*., 2016). GI helminths not only cause direct damages but also lower the immunity of the infected animals and predispose them to a wide array of diseases and even mortality.

As regards GI helminthes, strongylosis has been reported from almost all parts of world and estimated to affect >90% of equine population (Nielsen *et al*., 2006). Large strongyles like *Strongylus vulgaris*, *S. edentates* and *S. equinus* are generally recognized as the most important and pathogenic internal parasites in horses. However, small strongyles (also called cyathostomin) infections are generally mild in nature which may not produce any significant effects in the horses. Apart from the strongyles, other GI helminths of equines are pinworm (*Oxyuris equi*), stomach worms (*Habronema* sp.), tapeworms (*Anoplocephala* sp.) and amphistomes.

In Indian scenario, information is available on GI parasitism of horses from different regions excluding Punjab (Yadav *et al*., 2014; Matto *et al*., 2015; Adeppa *et al*., 2016). Regarding the Punjab state, there appears to be one published sporadic prevalence study conducted in Patiala district (Kaur and Kaur, 2008) and one from the central plain zone of Punjab (Singh *et al*., 2016) for determining the prevalence of GI parasites especially strongyles in horses. Hence, the present study was planned to assess the status of GI parasites in horses in southern districts of Punjab state and their association with various risk factors.

MATERIALS AND METHODS

Study area: In the present study, seven districts representing the southern Punjab *viz.* Barnala, Bathinda, Faridkot, Fazilka, Sri Muktsar Sahib, Mansa and Sangrur were selected. The climatic conditions of this region are sub-tropical, mostly dry and hot with annual rainfall varying from 300 to 600 mm in a short rainy season and the average maximum and minimum temperatures are 40–48°C and 2–7°C, respectively.

Sample collection: A total of 257 freshly laid faecal samples were collected randomly from horses of Barnala, Bathinda, Faridkot, Fazilka, Sri Muktsar Sahib, Mansa and Sangrur during one year study period (January 2016 to December 2016). After collection, each sample was labelled properly, kept in an ice box and transported to the Postgraduate Laboratory, Department of Veterinary Parasitology, College of Veterinary Sciences, GADVASU, Ludhiana for qualitative and quantitative examination of GI parasites. Other relevant information *viz*. sex, age, farm management system and season were also recorded at the

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

Risk factors	NE	NP	S (%)	PE (%)	M %)	A (%)	E (%)	T (%)	OP (%)
Season									
Rainy	120	39	21 (17.50)	7 (5.83)	4 (3.33)	4 (3.33)	2 (1.67)	1 (0.83)	32.50
Summer	42	07	7 (16.70)	0 (0.0)	0 0.0)	0 (0.0)	0 (0.0)	0 (0.0)	16.70
Winter	95	03	3 (3.15)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3.15
c^2 value									36.1506*
Sex									
Female	160	32	21 (13.12)	6 (3.75)	3 (1.87)	1 (0.625)	1 (0.625)	0 (0.0)	20.00
Male	97	17	10 (10.30)	1 (1.03)	1 (1.03)	3 (3.09)	1 (1.03)	1 (1.03)	17.52
c^2 value									5.7386
Age									
<2 year	56	12	9 (16.07)	2 (3.57)	0 (0.0)	1 (1.78)	0 (0.0)	0 (0.0)	21.40
2 -4 year	51	11	7 (13.72)	2 (3.92)	1 (1.96)	0 (0.0)	1 (1.96)	0 (0.0)	21.60
>4 year	150	26	15 (10.00)	3 (2.00)	3 (2.00)	3 (2.00)	1 (0.67)	1 (0.67)	17.33
c^2 value									12.7036
Farmtypes									
Organised	125	11	4 (3.20)	4 (3.20)	0 (0.0)	2 (1.60)	1 (0.80)	0 (0.0)	8.8
Unorganised	132	38	27 (20.40)	3 (2.30)	4 (3.03)	2 (1.51)	1 (0.75)	1 (0.75)	28.7
c^2 value									23.0998*
Total	257	49	31 (12.06)	7 (2.72)	4 (1.56)	4 (1.56)	2 (0.78)	1 (0.38)	19.06

NE: Number examined; NP: Number positive; S: Strongyles; PE: *Parascaris equorum*; M: Mixed infection (*Strongyloides westeri* + Strongyle sp.); A: Amphistome; E: *Eimeria* sp.; T: *Trichuris* sp.; OP: Overall prevalence

Superscript *indicates value varying at $P \leq 0.01$ (Chi-square value calculated by considering amphistome, *Eimeria* and *Trichuris* as one group)

time of sampling.

The effect of the various risk factors on the prevalence of GI parasitism were also determined *viz.* sex (male and female), age (<2 year; 2-4 year and >4 year), seasons (summer, monsoon and winter), and farm types (organized and unorganized).

Coproscopic examination: The collected samples were thoroughly screened macroscopically for colour, consistency, blood, mucus, parasites and/or segments, if any. Thereafter, the samples were subjected to qualitative coproscopic examination using direct smear, faecal sedimentation and floatation techniques for screening of parasitic eggs/oocysts (Soulsby, 1982). For quantitative faecal sample examination, McMaster's technique was used to estimate the eggs per gram (EPG) of faeces and the intensity of infection was categorized as per Soulsby (1982). Coproculture examination was also performed on representative number of strongyle positive faecal samples as per standard protocol (Soulsby, 1982). The hatched out larvae were harvested by Baermann's apparatus and identified under light microscope as per standard keys of MAFF (1986) and Kornas *et al*. (2009).

Statistical analysis: Possible associations between the evaluated variables and positive reaction to the agents

Fig. 1: L3 stages of Cyathostomes encountered during coproculture; Long and broad larvae (723±0.917 µm); Long filamentous sheath present; No. of intestinal cells: 8; Shape of intestinal cells: Triangular; Intestinal cells clear ; Arrangement of intestinal cells ; Type A: First 2 cells in double row and remaining 6 in single row; Type C: First 4 cells in double row and 4 in single row

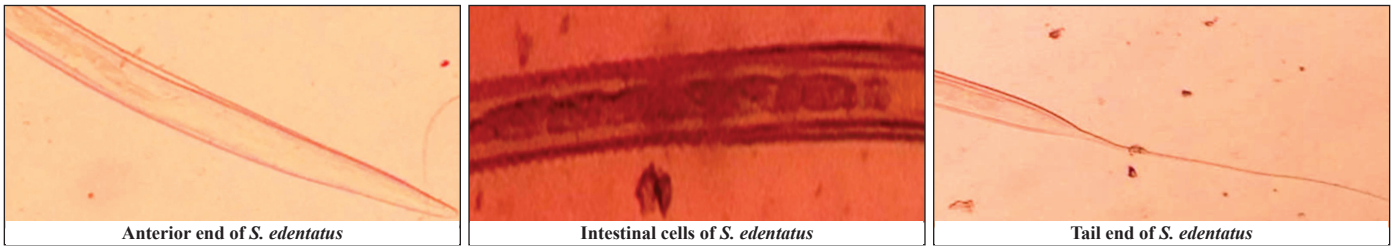

Fig. 2: L3 stages of Strongylus edentatus encountered during coproculture; Small and thin larvae (748±2.4 µm); Filamentous sheath present; No. of intestinal cells: 18-20 (poorly defined elongated cells); Intestinal cells not well defined

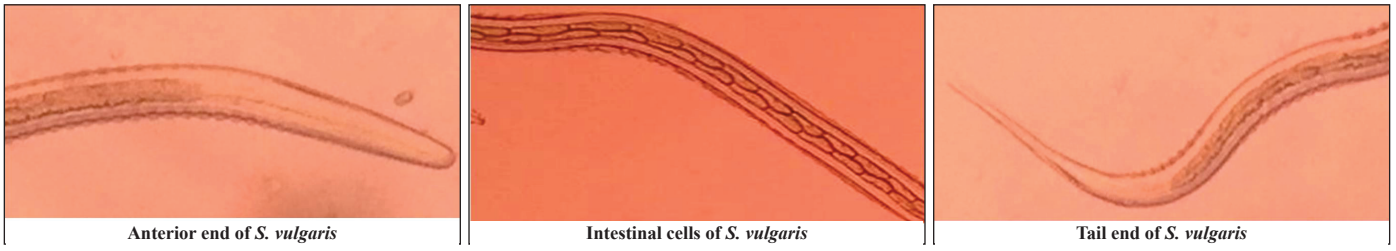

Fig. 3: L3 stages of Strongylus vulgaris encountered during coproculture; Long and broad larvae (927±4.06 µm); Filamentous sheath present; No. of intestinal cells: 28-32; Shape of intestinal cells: rectangular; Intestinal cells well defined

were determined by the Chi-square test. The effect of various risk factors on the prevalence of gastrointestinal parasitism was analysed by SAS software Version 9.3 (SAS Institute, CARY, USA).

RESULTS AND DISCUSSION

Coproscopic Analysis: The overall prevalence of GI parasites in horses recorded from the districts of southern Punjab in the present study was 19.06% with strongyle infection (12.06%) being the most predominant and *Trichius* sp. (0.38%) was the least encountered GI parasite (Table 1). Among trematodal infections, equine amphistomes were reported only with prevalence rate of 1.56%. The results of present study are in agreement with the observations of Singh *et al*. (2016) who reported 17.9% prevalence of GI parasites from Central Plain Zone of Punjab. However, many workers from various parts of India have reported comparatively higher prevalence rates of GI helminths from horses ranging from 20.63% to 84.0% (Kaur and Kaur, 2008; Yadav *et al*., 2014; Matto *et al*., 2015; Adeppa *et al*., 2016). The variations in the overall prevalence of GI parasites may be attributed to differences in managemental practices adopted in equine husbandry, geo-climatic conditions prevailing in the area selected for study, time of sampling and number of samples selected to undertake the investigation.

In the present study, strongyle infection was found to be the highest (12.06%) that is congruent with the findings of Adeppa *et al*. (2016) from Shimoga region, Karnataka state, India. The data obtained is more or less similar with the findings of Singh *et al*. (2016) who recorded the prevalence of strongyles as 17.90% from Central Plain Zone of Punjab.

The prevalence rate of *Parascaris equorum* (2.72%) recorded in the present study is comparatively lower than that of various workers worldwide, including India who reported the prevalence rates to vary from 7.14% to 84.61% (Kaur and Kaur, 2008; Ionita *et al*., 2013; Adeppa *et al*., 2016) and in close proximity with that of Singh *et al*. (2012). Among trematodal infections, amphistome was recorded as 1.56% which is in agreement with the studies of Matto *et al*. (2015) who reported 1.38% prevalence of amphistome infection. The lower prevalence rates of the amphistomes in the current study might be attributed to the fact that the study was carried out in districts of southern Punjab where the geo-climatic conditions are typically hot and dry and the soil type is loamy-sandy, and these factors are not favourable for survival and maintenance of snails, hence, resulting in lower prevalence of amphistomes.

The quantitative analysis of faecal samples

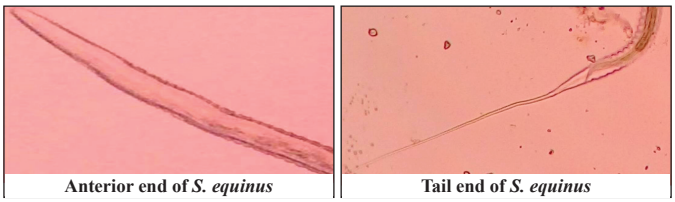

Fig. 4: L3 stages of Strongylus equinus encountered during coproculture; Long and thin larvae (858±2.6 µm); Sheath present; No. of intestinal cells: 16 (poorly defined); Shape of intestinal cells: Rectangular; Intestinal cells not clear

positive for strongyles revealed eggs per gram (EPG) of faeces ranging from 50 to 1500 (441.93±61.5) thereby showing mild to severe intensity of infection (Soulsby, 1982). The EPG of *P. equorum* infection ranged from 100 to 200 (142.85± 17.0) indicating mild type of infection (Soulsby, 1982). Regarding the intensity of infection, Anutescu *et al*. (2016) reported the EPG values of strongyles to vary from 50 to 2450 with an overall mean output as 417.5±721.2 which is in close proximity to that obtained in the current study. In previous study from Punjab, Singh *et al*. (2016) reported rare to mild type of strongyle infection with EPG ranging from 50 to 500 with an average of 85.71 ± 21.07 in horses of Central Plain Zone. Regarding *P. equorum*, Ionita *et al*. (2013) reported the intensity rate varying from 75 to 1200 which is higher than obtained in the present study. This variation might be due to variations in the prevalence rates for respective parasites, age groups, climatic conditions, sample size, sampling period, availability of veterinary services, and the importance value of animals to the owners.

The coproculture studies revealed highest proportion (62.5%) of small strongyles larvae (cyathostomes), of which maximum (48.2%) were identified as type A (Fig. 1), 41.4% as type C (Fig. 1) while 10.4% could not be identified. Regarding the large strongyles (34.8%), *Strongylus edentatus* was the predominant species as 15.7% (Fig. 2) followed by *S. vulgaris* as 10.4% (Fig. 3) and *S. equinus* was least encountered as 8.7% (Fig. 4). Cyathostomes are predominant species parasitizing horses as reported globally by many workers (Anutescu *et al*., 2016; Singh *et al*., 2016). The most probable reason behind this may be the fact that the anthelmintics have been found to be less effective against cyathostomes than large strongyles because of the fact that routine doses of modern anthelmintics often fail to eradicate encysted third- and fourth-stage larvae of cyathostomes (Xiao *et al*., 1994).

Assessment of various risk factors on prevalence of GI parasites: The details of effect of various risk factors on the prevalence of GI parasites of horses of southern Punjab districts are presented in Table 1. Amongst various risk factors, season was reported as a major factor for variation in the prevalence of the GI parasites and the data was statistically highly significant (P<0.01). Highest prevalence was recorded in rainy (32.5%) followed by summer (16.7%) and least in winter (3.15%) season. Sex-wise higher prevalence (20%) in females as compared to males (17.52%) was recorded but the variation was statistically non-significant. Age-wise prevalence revealed higher rate (21.6%) of infection in adults followed by young (21.4%) and least in old animals (17.33%), respectively, but the data was statistically non-significant. Farm type was also found to be a predominant risk factor for prevalence of the GI parasites (unorganised farm- 28.7% and organised farm- 8.8%) (P<0.01).

In the current study, seasonal prevalence was in close proximity with the observations of Singh *et al*. (2016) who also reported the prevalence of GI infections to be maximum in rainy followed by summer and least in winter from Central Plain Zone of Punjab. Many workers from various parts of world including India have reported more or less similar seasonal trend regarding the prevalence of GI parasites in equines (Saeed *et al*., 2010; Maria *et al*., 2011; Matto *et al*., 2015). The highest prevalence rate recorded in rainy (monsoon) season could be because of optimal ambient temperature and high relative humidity conducive for the development of free living pre-parasitic stages of helminths (Saeed *et al*., 2010, Maria *et al*., 2011). However, as cold and dry conditions are comparatively unfavourable for the development of parasitic stages, this leads to lower infection rates in these seasons.

In the present study, it was observed that female animals are more susceptible for strangles infection as compared to males. Similar findings have also been reported by Singh *et al*. (2016) from Central Plain Zone of Punjab. The findings of age-wise prevalence in the present study may be due to the lower immunity in <2 year and 2-4 year age group whereas, the least prevalence rate was reported in >4 year age group may be due to the care provided to this age group due to its maximum value in different related fields. On similar lines, Singh *et al.* (2016) have reported higher prevalence rates in animals up to 4 years of age than those >4 years from Central Plain region of Punjab.

Regarding farm type, the results of current study are on similar lines with those of many workers (Sharma *et al*., 2011; Yadav *et al*., 2014) who reported higher prevalence in unorganized sector in comparison of organized sector. Among the predisposing factors of internal parasite infections *viz*. climate, nutritional deficiency, grazing habits, immunological status, pasture management, presence of intermediate host/vectors, the number of infective larvae/eggs in the environment, damages inflicted to the health and productivity including

loss in body weight, poor reproductive performance, digestive disturbance and emaciation for longer period (Radostits *et al*., 1994) are greatly influenced by the managemental practices adopted in organised farms and have huge impact on prevalence of GI parasites as compared to the unorganised farms.

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