ASSESSMENT OF RISK FACTORS ASSOCIATED WITH PREVALENCE OF GASTROINTESTINAL HELMINTHS IN BUFFALOES FROM PUNJAB STATE, INDIA

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ABSTRACT

A total of 956 faecal samples were collected from buffaloes representing 21 districts of different agro-climatic zones of the Punjab state, India during April, 2013 to May, 2014 for detection of various gastrointestinal helminth (GIH) parasites. Coprological examination of the samples revealed an overall prevalence of 43.20% for GIH with mean egg per gram of faeces ranging from 170±7.07 to 226±7.14. The parasites recorded with their respective prevalences (%) in the descending order were: strongyles (27.62%), amphistomes (16.94%), *Fasciola* spp. (5.23%), *Moniezia* spp. (4.18%), *Trichuris* spp. (2.2%), *Strongyloides* spp. (1.88%) and *Capillaria* spp. (0.10%). The results of multivariate analysis showed that prevalence of GIH in buffalo population was associated with various risk factors *viz*. districts (P=0.000; OR: 0.474; CI 95%: 0.182-1.234), agro-climatic zones (P=0.000; OR: 1.156; CI 95%: 0.64-2.082) and seasons (P=0.316; OR: 1.138; CI 95%: 0.838-1.545). The maximum prevalence of GIH infection was recorded in monsoon season (47.84%) and lowest in winters (39.76%) with non significant differences (P>0.05). Agro-climatic zone-wise prevalence of GIH revealed the highest prevalence in undulating plain zone receiving maximum annual rainfall (62.88%) and lowest in western plain zone with minimum annual rainfall (33.33%) and the differences were highly significant (P<0.01). Coproculture analysis of the strongyle positive samples revealed the presence of larvae of genera *Oesophagostomum*, *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Chabertia* and *Strongyloides* in decreasing order of their prevalence. The data generated could be of immense help in formulation of effective strategies for gastrointestinal parasite control in different agro-climatic zones of Punjab.

Keywords: *Bubalus bubalis*, buffalo, agro-climatic zones, coproculture, epidemiology, gastrointestinal helminths, risk factors, Punjab

INTRODUCTION

The buffalo population is estimated to be 185.29 million worldwide, of which India contributes approximately 56.7% with 105.1 million heads (FAO, 2008). The state of Punjab has been one of the front runners in white revolution in the country and stands 5th in milk production amongst all the Indian states with buffalo population of 5.159 million (DAHD, 2014). Buffaloes have been raised as economically important animals because of their multipurpose

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utility in terms of providing milk, meat and good quality hides. Furthermore, they are also used as draft animals ("tractors" in Southeast Asia) in agriculture farms, means of transportation, and their dung act as a good fertilizer (Liu *et al*., 2009). Gastrointestinal helminth (GIH) infections of buffaloes are common, which result in considerable economic losses to the buffalo industry and farming communities as a consequence of deaths of infected animals, reduced weight gains and milk production as well as condemnation of the affected organs after slaughter (Roberts and Fernando 1990; Capuano *et al*., 2006; Raza *et al*., 2007). Despite significant production losses, which may run into millions of rupees, the problem of gastrointestinal (GI) parasitism is invariably neglected because of its chronic and insidious nature (Jithendran and Bhat, 1999).

The prevalence of GIH in buffaloes has been well documented from different parts of world (Afridi *et al*., 2007; Mamun *et al*., 2011; Saha *et al*., 2013; Gorish *et al*., 2014), including India (Jithendran and Bhat, 1999; Muraleedharan, 2005; Gupta *et al*., 2012; Jamra *et al*., 2014) along with few sporadic reports from the Punjab state (Haque *et al*., 2011; Singh *et al*., 2012; Jyoti *et al*., 2013). Various risk factors, including those of the host and environment, play an important role in the onset of GIH infections in animals. The effects of GIH infections on production of particular livestock species depend mostly upon the age of the animal, genotype, parasite species involved and intensity of the worm population (Al-Shaibani *et al*., 2008). The climate in a particular locality is also one of the important factors that determines the type and severity of parasitic infections in animals (Tariq *et al*., 2010). However, till date, studies on the risk factors associated with the prevalence of GIH are lacking in buffalo population of the Punjab state, India. Therefore, the present study was undertaken with the aim to determine the prevalence and assessment of associated risk factors of GIH infections in buffalo population of various agro-climatic zones of the Punjab state.

MATERIALS AND METHODS

Study area

The state of Punjab extends from the latitudes 29^{o}30' N to 32^{o}32' N and longitudes 73^{o}55' E to 76^{o}50' E in the northwest region of India. It covers a geographical area of 50,362 sq. km which is 1.54% of country's total area and lie between altitudes 180 meters and 300 meters above mean sea level. Average rainfall in Punjab is 565.9 mm and ranges from about 915 mm in north to 102 mm in south (http://punjabonline.in/Profile/Geography/climate.asp). The temperature starts rising in February but actual summer season starts from April and lasts till June, whereas, monsoon begins in July and culminates in September with varied rainfall in different areas. In Punjab, winter starts from October and the weather becomes chilly and frosty from December to February. The prevailing environmental conditions of the region provide nearly an ideal environment for survival of the pre-parasitic free living stages of GIH. Further, the state of Punjab has been classified into five agro-climatic zones on the basis of homogeneity, rainfall pattern, distribution, soil texture, cropping patterns etc.

Buffaloes were examined from twenty one districts falling under five major agro-climatic zones of the Punjab state *viz*. sub-mountain undulating zone with average annual rainfall of>900 mm (Districts: Gurdaspur, Hoshiarpur), undulating plain zone: 800 to 900 mm (Mohali, Ropar and

SBS Nagar), central plain zone: 500 to 800 mm (Amritsar, Fatehgarh Sahib, Jalandhar, Kapurthala, Ludhiana, Patiala and Tarn Taran), western plain zone: 400 to 500 mm (Fazilka, Faridkot and Ferozepur) and western zone: <400 mm (Barnala, Bhatinda, Mansa, Moga, Muktsar and Sangrur). Among these zones western and western plain are the hottest and dry zones of Punjab whereas sub-mountain undulating and undulating plain receive maximum rainfall and have moderate humid climate.

Collection of samples

A total of 956 faecal samples from adult female buffaloes in 21 districts of different agro-climatic zones of Punjab were randomly collected directly from the rectum during April, 2013 to May, 2014. Collection of samples was repeated seasonally, with the aim to demonstrate the seasonal differences in prevalence. The samples were placed in sterile polythene bags and labelled carefully indicating the host's detail, location and time of collection, kept in a cool transport box and brought to the Postgraduate Laboratory, Department of Veterinary Parasitology, COVS, GADVASU, Ludhiana for further examination.

Faecal analysis and egg counts

The faecal samples were first subjected to standard qualitative examination using direct smear method and concentration techniques (floatation and sedimentation) for detection of the helminthic eggs. The eggs were identified on the basis of the morphological features as described by Soulsby (1982). The samples positive for helminthic eggs were subjected to quantitative faecal sample examination employing standard McMaster's and Stoll's dilution technique to calculate the eggs per gram (EPG) of faeces in order to assess the severity of infection (Soulsby, 1982).

Coproculture

A representative number of faecal samples positive for strongyle eggs were pooled in equal quantities and used in coproculture at 27^{o}C as per standard protocol. The hatched out larvae were harvested and used for identification (Van Wyk and Mayhew, 2013).

Statistical analysis

All data analyses were performed by using statistical software program (SPSS for Windows, Version 19.0, USA). Association between the prevalence of GI helminth infections and various factors was carried out by Chi square (χ^2-test). Variables with significant association at $P<0.05$ (two-sided) were subjected to the multivariate logistic regression model. The results were each expressed as P-value and odds ratio (OR) with a 95% confidence interval (CI 95%).

RESULTS AND DISCUSSION

Of the total 956 samples examined, 413 were found positive for GIH showing an overall prevalence as 43.20%. Similar findings regarding the prevalence of GIH infections in buffaloes have been reported earlier from the same region (Haque *et al*., 2011; Jyoti *et al*., 2013) as well as other states of India, *viz*. Madhya Pradesh and Karnataka (Kashyap *et al*., 1997; Jagannath *et al*., 1988).

However, many workers from India as well as abroad have reported higher prevalence rates of GIH in buffaloes (Azam *et al*., 2002; Singh *et al*., 2006; Iqbal *et al*., 2007; Kaur and Kaur, 2008; Gupta *et al*., 2011; Mamun *et al*., 2011; Gupta *et al*., 2012; Zaman *et al*., 2014; Gorsich *et al*., 2014).

The lower prevalence rate in the present study thus may be possibly due to different geographical area, sample size as well as adoption of improved animal husbandry practices, animal welfare and rational use of anthelmintics in this part of the country (Singh *et al*., 2012).

Though considerable infections were of mixed type (18.72%), the most predominant helminth infection was of strongyles (27.62%) which is congruent with the findings various workers of India and abroad (Azam *et al*., 2002; Murleedharan, 2005; Haque *et al*., 2011; Singh *et al*., 2012; Jyoti *et al*., 2013; Zaman *et al*., 2014). The higher prevalence of strongyles in buffaloes can be attributed to the fact that the warm and wet environment favoured by buffaloes also proves to be favourable for the development of pre-parasitic free living stages of these parasites (Sreedhar *et al*., 2009). Also, the feeding habits and liking of unhygienic habitats of buffaloes (Bilal *et al*., 2009) contribute heavily towards the increased risk of exposure to the GI parasites. Moreover, various workers have reported amphistomes as the most prevalent GI parasite in buffalo population from different parts of India (Yadav *et al*., 2007; Gupta *et al*., 2011; 2012) which is in contrast to the findings of present study which may be due to the fact that the animals of this region are mostly stall fed thus decreasing chances of exposure to the infective metacercarial stages of the parasite encysted on the vegetation in close vicinity of water bodies (Singh *et al*., 2012). Other helminths recorded were *Fasciola* spp. (5.23%), *Moniezia* spp. (4.18%), *Trichuris* spp. (2.2%), *Strongyloides* (1.88%) and *Capillaria* spp. (0.10%) (Table 1).

Parasite egg and oocyst counts reflect the ability of host to regulate the survival/expulsion (Balic *et al*., 2002), growth, or reproduction (Rowe *et al*., 2008) of parasites to which it has been exposed. The quantitative examination of positive faecal samples revealed mean EPG of 172±15.62, 226.92±7.14 and 170.0±7.07 for strongyles, amphistomes and *Fasciola* spp., respectively (details in Table 2). The findings regarding the severity of GIH parasitism, particularly, strongyle, amphistome and *Fasciola* spp. in buffaloes in the present study is on similar lines with that of Mamun *et al.* (2011). However, few studies from different geographical locations of India have reported comparatively higher EPG counts to the findings of present study (Jithendran and Bhat, 1999; Wadhwa *et al*., 2011; Jamra *et al*., 2014). The findings of comparatively lower EPG counts may be possibly due to better managemental and husbandry practices prevalent in the state (Singh *et al*., 2012). However, whether, the lower worm load in buffaloes is due to lower establishment and/or lower fecundity of strongyle nematodes needs to be investigated.

The strongyle positive samples when subjected to coproculture revealed the presence of larval stages of the genera of *Oesophagostomum* (32%), *Haemonchus* (27%), *Trichostrongylus* (17%), *Cooperia* (11%), *Chabertia* (8%) and *Strongyloides* (5%) in decreasing order of prevalence. Various researchers in their respective studies on buffalo population have encountered these nematodes from abroad (Raza *et al*., 2007; Zaman *et al*., 2014) different parts of India (Jithendran and Bhat, 1999; Murleedharan, 2005; Jamra *et al*., 2014).

Seasonal dynamics of GI helminths

The current study indicated that season plays an important role in the prevalence of GIH. A non-significant association between the prevalence of GIH and the season was observed (P=0.316; OR: 1.138; CI 95%: 0.838 to 1.545). A β-value of -0.101 was recorded between the prevalence of GIH and the various seasons (summer followed by monsoon and winter), thus indicating a decrease in prevalence with a decrease in ambient temperature (Table 3). Based on a nationwide survey on epidemiology of parasites in dairy animals from seven different agro-climatic zones, Sanyal and Singh (1995) indicated an increased parasite burden in the host as well as on pasture during the rainy season. Similarly, in the present study, the highest infection rate was recorded in monsoon (47.84%), followed by summer (42.90%) and least in winter (39.76%) (Table 1). The seasonal fluctuation of the year had a non-significant effect on the prevalence of GIH infections in buffaloes which are identical to the findings of several workers (Jithendran and Bhat, 1999; Mamun *et al*., 2011; Jyoti *et al*., 2011; Gupta *et al*., 2012; Saha *et al*., 2013). The most prevalent helminth infection in buffalo population of the state was strongyles (31.29%) with maximum prevalence as well as severity of the infection (191.67±24.01) were observed in rainy season (Table 1). These findings may be attributed to the fact that rainy season provides optimum conditions of high humidity and ambient temperature required for development of pre-parasite/infective stages such as eggs, cysts and oocysts, whereas, cool and dry conditions were comparatively unfavourable leading to much longer time taken by the parasitic species for development in the environment. Also, reduced immune-tolerance in rainy season may be responsible for higher incidence during this season (Murleedharan, 2005). Infection with *Capillaria* spp. (0.10%) was recorded in winter season which appears to the first report from the state. Regarding the trematodal infections, maximum prevalence as well as severity of the infection based on the EPG counts for amphistomes (226.09±20.08) and *Fasciola* spp. (177.27±17.74) were found to be the highest in summer (Table 2) which may be due to the long pre-patent periods of these infections. Furthermore, the EPG counts for amphistomes were higher than those for *Fasciola* spp. which may be due to the fact that the biological potential of intermediate host (snail) responsible for transmission of amphistome infection is high (Mage *et al*., 2002).

Zone-wise prevalence of GI helminths

Agro-climatic zones of the Punjab state, India, were found to be significantly associated with the prevalence of GIH (P<0.000; OR: 1.156; CI 95%: 0.640 to 2.082). A negative correlation (β = –0.670) (Table 3) was recorded between the prevalence of GIH and various agro-climatic zones, with drier zones of western region (western and western plain zone) revealing lower prevalence rates of GIH as compared to the zones receiving high annual rainfall (sub-mountain zone) (Table 1). These findings may be attributed to the fact that in the zones receiving high annual rainfall optimum conditions of humidity and temperature required for development of pre-parasite/infective stages prevails for comparatively longer duration than those of the drier zones. As regards the parasitic helminth concerned, strongyles were found to be the most predominant ones from all the agro-climatic zones of the region (Table 1). The EPG counts of the strongyle (250.00±150.02) and amphistome (275.00±42.96) infection were found to be highest in western plain zone. These results may be attributed to the fact that though this zone is a comparatively drier zone, the micro environment of the animal sheds may provide the optimal conditions (*viz*. moisture and temperature) for the development of pre-parasitic free living stages of strongyles due to the presence of kuccha flooring and poor drainage facilities (Haque *et al*.,

Table 1. Season and Zone wise prevalence of gastro-intestinal helminths in buffaloes of Punjab.

Risk factors		Number of samples (% prevalence)									
		Examined	Positive	Mixed	Strongyle	Amphistome	Fasciola	Moniezia	Strongyloides	Trichuris	Capillaria
Season	Summer	331	142 (42.90)	64 (19.33)	93 (28.10)	76 (22.96)	26 (7.90)	15 (4.53)	7 (2.11)	8 (2.42)	0
	Rainy	278	133 (47.84)	69 (24.82)	87 (31.29)	46 (16.54)	14 (5.03)	12 (4.70)	6 (2.20)	8 (2.88)	0
	Winter	347	138 (39.76)	46 (13.27)	84 (24.21)	40 (11.53)	10 (2.88)	13 (3.75)	5 (2.31)	5 (1.44)	1 (0.29)
	χ^2 value		4.117	13.686**	3.937	15.779**	8.483**	0.278	0.577	1.597	1.757
Agro-climatic zones	Sub-mountain	72	29 (40.28)	12 (16.67)	25 (34.72)	11 (15.28)	1 (1.39)	3 (4.17)	1 (1.39)	0	0
	Undulating plain	132	83 (62.88)	26 (19.70)	58 (43.94)	28 (21.21)	12 (9.09)	3 (2.27)	4 (3.03)	5 (3.79)	1 (0.76)
	Central plain	547	228 (41.68)	106 (19.38)	137 (25.05)	97 (17.73)	30 (5.48)	26 (4.75)	9 (1.67)	15 (2.74)	0
	Western plain	72	24 (33.33)	13 (18.06)	14 (19.44)	12 (16.67)	3 (4.17)	1 (1.39)	2 (2.78)	0	0
	Western	133	49 (36.84)	22 (16.54)	30 (22.56)	14 (10.53)	4 (3.01)	7 (5.26)	2 (1.50)	1 (0.75)	0
	Total	956	413 (43.20)	179 (18.72)	264 (27.62)	162 (16.94)	50 (5.23)	40 (4.18)	18 (1.88)	21 (2.2)	1 (0.10)
	χ^2 value		26.644**	0.874	25.331**	5.989	7.674	3.434	1.618	6.813	6.249

*P<0.05; **P<0.01

Table 2. Season and Zone wise EPG (Mean± S.E.) of gastro-intestinal helminths in buffaloes of Punjab.

Parasite	EPG (Mean±S.E.)							
	Season			Agro-climatic zones				
	Summer	Rainy	Winter	Sub-mountain	Undulating plain	Central plain	Western plain	Western
Strongyle	164.29±24.83	191.67±24.01	141.67±19.32	166.67±36.61	185.71±55.24	162.07±17.47	250.00±150.02	183.33±30.72
Amphistome	226.09±20.08	180.00±24.32	183.33±29.77	216.67±47.71	216.67±25.94	186.96±19.72	275.00±42.96	160.00±35.87
Fasciola	177.27±17.74	144.44±16.62	154.54±20.71	300*	200±23.60	140.00±11.15	200.00±47.14	125.00±21.65

*Only one case

Table 3. Final logistic regression model for factors associated with the prevalence of helminth infections in buffaloes.

Variables	Regression coefficient (β)	Standard error (SE)	P-value	Odds ratio (OR)	Confidence interval (95%)
Zone	-0.670	0.188	0.000	1.156	0.640-2.082
Season	-0.101	0.100	0.316	1.138	0.838-1.545
District	3.253	0.385	0.000	0.474	0.182-1.234

Table 4. District wise prevalence of gastro-intestinal helminths in buffaloes of Punjab.

Districts	Number of samples (% prevalence)									
	Examined	Positive	Mixed	Strongyle	Amphistome	Fasciola	Moniezia	Strongyloides	Trichuris	Capillaria
Amritsar	29	15 (51.72)	6 (20.69)	9 (31.04)	3 (10.34)	1 (3.45)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bathinda	31	7 (22.51)	4 (12.90)	5 (16.13)	2 (6.45)	1 (3.23)	2 (6.45)	0 (0.0)	0 (0.0)	0 (0.0)
Barnala	19	12 (63.16)	3 (15.79)	8 (42.11)	4 (21.05)	1 (5.26)	1 (5.26)	0 (0.0)	1 (5.26)	0 (0.0)
Faridkot	37	13 (35.13)	8 (21.62)	9 (24.32)	6 (16.22)	1 (2.70)	1 (2.70)	2 (5.41)	0 (0.0)	0 (0.0)
Fatehgarh Sahib	51	27 (52.94)	14 (27.45)	8 (15.69)	18 (35.29)	9 (17.65)	3 (5.89)	1 (1.97)	1 (1.97)	0 (0.0)
Fazilka	11	3 (27.27)	1 (9.09)	2 (18.18)	1 (9.09)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ferozepur	24	8 (33.33)	4 (16.67)	3 (12.50)	5 (20.83)	2 (8.33)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gurdaspur	10	4 (40.0)	2 (20.0)	3 (30.0)	2 (20.0)	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hoshiarpur	62	25 (40.32)	10 (16.13)	22 (35.48)	9 (14.51)	1 (1.61)	1 (1.61)	1 (1.61)	0	0
Jalandhar	25	7 (28.0)	6 (24.0)	5 (20.0)	3 (12.0)	1 (4.0)	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Kapurthala	12	3 (25.0)	1 (8.33)	2 (16.67)	1 (8.33)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ludhiana	328	147 (44.81)	64 (19.51)	91 (27.74)	51 (15.55)	11 (3.35)	18 (5.49)	6 (1.83)	10 (3.05)	0 (0.0)
Mansa	16	6 (37.50)	2 (12.50)	6 (37.50)	2 (12.50)	0 (0.0)	2 (12.50)	0 (0.0)	0 (0.0)	0 (0.0)
Moga	30	16 (53.33)	9 (30.0)	11 (36.70)	1 (3.33)	0 (0.0)	2 (6.67)	0 (0.0)	0 (0.0)	0 (0.0)
Mohali	15	10 (66.67)	4 (26.67)	10 (66.67)	3 (20.0)	1 (6.67)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Muktsar	20	4 (20.0)	2 (10.0)	4 (20.0)	2 (10.0)	1 (5.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
Patiala	78	21 (26.92)	11 (14.10)	17 (21.79)	15 (19.23)	6 (7.69)	5 (6.41)	1 (1.23)	4 (5.12)	0 (0.0)
Ropar	28	8 (28.57)	4 (14.29)	6 (21.43)	2 (7.14)	0 (0.0)	2 (7.14)	0 (0.0)	0 (0.0)	0 (0.0)
SBS Nagar	89	65 (73.03)	18 (20.22)	42 (47.19)	23 (25.84)	11 (12.36)	1 (1.12)	4 (4.49)	5 (5.62)	1 (1.12)
Sangrur	17	4 (23.53)	2 (11.76)	2 (11.76)	3 (17.64)	1 (5.88)	0 (0.0)	1 (5.88)	0 (0.0)	0 (0.0)
Tarn Taran	24	8 (33.33)	4 (16.67)	5 (20.83)	6 (25.0)	2 (8.33)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
χ^2 value		74.366**	12.825	49.443**	37.129*	37.819**	24.300	13.863	18.705	9.752

*P<0.05, **P<0.01

2011). Among the trematodes, amphistomes were reported to be the most prevalent (16.94%) in the region with a similar prevalence pattern as reported in strongyle infection (Table 1) which may be due to the higher biotic potential of intermediate host as well as the higher proportion of animals shedding the eggs in all management systems, zones, farms and age groups might be the probable reason (Keyyu *et al*., 2005). Simultaneously, the western zone (5.26%) and central plain zone (4.75%) reported a comparatively higher prevalence of *Moniezia* spp. infections.

Prevalence of GI helminths in different districts

A highly significant (P=0.000; OR: 0.474; CI 95%: 0.182 to 1.234) association of the districts and prevalence of GIH was recorded with a positive correlation (β=3.253) (Table 3). In the present study, highest infection rate for GIH (73.03%) was recorded from district SBS Nagar (Table 4). As far as the parasitic helminths are concerned, maximum prevalence of strongyles, amphistomes, *Fasciola* spp. and *Moniezia* spp. was recorded from district Mohali (66.67%), Fatehgarh Sahib (35.29%), Fatehgarh Sahib (17.65%) and Gurdaspur (20.0%), respectively (Table 4). Information on prevalence and distribution on the GIH in buffaloes of Punjab state is either fragmentary or sporadic (Kaur and Kaur, 2008; Haque *et al*., 2011; Singh *et al*., 2012; Jyoti *et al*., 2013). To our best of knowledge, till date, studies on the assessment of risk factors associated with the prevalence of GIH and coprocultural evaluation of the strongyle worms in buffalo population are lacking. The data generated in the present study in this regard could be of immense help in formulation of effective strategies for GI parasite control in different agro-climatic zones of Punjab.

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