CROSS-SECTIONAL SURVEY OF HELMINTHIASIS IN BUFFALOES AT TEHSIL JATOI AND TEHSIL MUZAFFAR GARH, SOUTHERN PUNJAB, PAKISTAN

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ABSTRACT

In the four year study from 2009 to 2013, a total of 500 faecal samples from buffaloes from different locations of Tehsil Jatoi and Tehsil Muzaffar Garh, Southern Punjab were analyzed to confirm the presence of gastrointestinal parasitic infection. The recovered parasites were five nematodes viz Toxocara vitulorum (16.6%), Oesophagostomum radiatum (3.2%), Bunostomum phlebotomum (1.6%), Cooperia spp. (1.6%), *Trichostrongylus* spp. (0.8%). The two trematodes were Fasciola hepatica (8.4%), Paramphistomum cervi (15%). Age-wise prevalence was 79.5% and 47% in buffalo calf and adult buffalo, while sex-wise prevalence was 78.4% and 50.93% in male and female buffalo, respectively. Chi-square statistical design was applied to data to know the dependence of helminth's prevalence on sex and age of animals.

Keywords: buffaloes, helminths, southern Punjab, Pakistan

INTRODUCTION

Helminths are recognized as a major constraint to livestock production throughout the

world (Ibrahim et al., 1984 and Waler et al., 1987). water buffaloes are considered common host for helminthiasis in tropical and sub-tropical area. Helminthiasis inflicts huge economic losses even deaths of infected animals. The economic losses are in a variety of ways like lowered the fertility, reduction in work capacity, involuntary culling of emaciated animals, reduction in food intake, lesser weight gains, lower milk production, huge treatment costs, and mortality in heavily parasitized animals (Lebbie and Irungu, 1994), the other can be reduced weight gains and the condemnation of infected organs at slaughter (Liu et al., 2009). Prevalence of Gastrointestinal Tract helminthes in ruminants has been reported up to 25 to 92% in different areas of Pakistan by various workers like Ali et al., 2000; Raza et al., 2007; Ijaz et al., 2008; Al-Shaibani et al., 2008; Kakar and Kakar-sulemankhel, 2008; Raza et al., 2010; Ayaz et al., 2013 and Igbal et al. (2002) has pointed out various parasitic problems like Facioliasis, hydatidosis, coccidiosis, theileriosis and babesiosis as the major parasitic problems of ruminants in Pakistan in the order of priority. A number of factors that influence the prevalence of helminthes that includes age (Mckenna, 1981), sex (Asanji and Williams, 1987), breed (Miller, 1998; Mirza and Razzak, 1998), worm population (Ankers et al., 1997), weather condition (Asanji

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and Williams, 1987, Mohiuddin *et al.*, 1984) and husbandry or managemental practices (Tan *et al.*, 1996). Some helminths infecting buffaloes primarily has zoonotic importance such as schistosomiasis, cycstic echinococcosis, and fasciolosis (Liu *et al.*, 2009; Cringoli *et al.*, 2007). Punjab province in Pakistan has largest population of buffalo so considering the health implications and the economic potential of water buffaloes, the issue of investigating parasitic infections of buffaloes is of relevance (Rinaldi *et al.*, 2007; Veneziano *et al.*, 2007) for the cross sectional study.

MATERIALS AND METHODS

The present cross sectional study was initiated from January 2009 to June 2013 to determine the point prevalence of Gastrointestinal tract helminthiasis in the buffalo under field conditions at Tehsil Jatoi and Tehsil Muzaffar Garh, Southern Punjab, Pakistan.

Study area

From January 2009 to June 2013, fecal sample of 500 buffaloes (Between age ranging six months to two years and from three years to six years and Sex-wise) were brought to the Laboratory of Veterinary Parasitology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan for presence of GIT parasites. The study area is located at latitude 288570 °N to 308460 °N and longitude 708300 °E to 718470 °E.

Sample collection

Faecal samples of buffaloes (n=500) were randomly collected in sterile polythene bags directly (Ayaz, 2010). The faecal samples were brought to the Laboratory of Veterinary Parasitology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan for identification of eggs/larvae or adult helminths.

Sample analyses

Fecal samples were examined for helminthes eggs/larvae/adult by using direct and indirect techniques (Ayaz, 2010) and for identification of certain nematodes, copro-culture/ Baermann method (Ayaz, 2010) were performed to obtain larval stages. Both eggs and larvae from copro-culture were identified by using standard techniques as described by MAFF (1979) and Soulsby (1982). Briefly, one gram of fecal sample was mixed well in a drop of water and a relatively homogenous and transparent preparation was obtained and examined under microscope by placing a drop of suspension on slide with cover slip. At least three direct smears were examined from each sample. All the samples were also examined by using concentration techniques, i.e. floatation and sedimentation. For floatation technique, five grams of feces was mixed in 50 ml of water and strained through a sieve no. 60 micron mesh to remove the course material. The mixture was allowed to sediment for half an hour. The supernatant was discarded and sediment was mixed with saturated salt solution. The suspension was centrifuged at 1000 rpm for two minutes. The upper 0.1 ml of centrifuged suspension was transferred to a glass slide and examined under microscope at 10 X for the presence of helminthes eggs. More over a relatively new technique for "a single slide positive sample" was also developed as "micro-floatation technique" (Ayaz, 2010). For sedimentation technique to examine heavy eggs, five grams of faeces was mixed in 50 ml of water and strained through a sieve mesh no. 60 micron to remove the course material. The mixture

was allowed to sediment for half an hour. After centrifugation, the supernatant was decanted and washing was continued until supernatant became clear. A drop of 0.1 ml was taken from sediment with the help of Pasteur's pipette on slide and was examined under microscope at 10 X for the presence of helminthes eggs.

Feco-Copro-Culture

Feco-Copro-Culture provides an environment suitable for hatching and development of helminths eggs. Samples found positive for nematode eggs were broken up finely, using either a large pestle and mortar or spatula and were placed in a glass jar or petri-dish for incubated at 26°C for 3-7 days. After incubation, samples were examined for the presence of larvae and were identified with the help of key by MAFF (1979).

Statistical analyses

Data on the prevalence of helminthiasis was analyzed using Chi-square statistical design and percentage on the basis of sex and age. Graphical representation of tabulated data was also done.

RESULTS

The present cross sectional study was commenced from January 2009 to June 2013 to determine the prevalence of GIT helminthes in buffaloes. Overall Age-wise and sex-wise prevalence in buffalo was 60% and 57.8%, respectively. The highest prevalence for nematodes (119/500; 23.2%) followed by trematodes (117/500;23.4%) was recorded. A total seven species of helminths including five species of nematodes, vitulorum, i.e. Toxocara Oesophagostomum radiatum, Bunostomum phlebotomum, Cooperia spp., Trichostrongylus spp. and two trematodes species i.e. Fasciola hepatica, Paramphistomum cervi were recorded. Among various species of helminthes Toxocara vitulorum was the most prevalent species of helminthes which was followed by Paramphistomum cervi, Fasciola hepatica, *Oesophagostomum* radiatum, **Bunostomum** phlebotomum, Cooperia spp. and Trichostrongylus species respectively. The mixed helminthes infections (52/500; 12.4%) was often composed of 07 species including Toxocara vitulorum, *Oesophagostomum* radiatum, Bunostomum phlebotomum, Cooperia spp., Trichostrongylus

Table 1. Prevalence of different species of helminths in buffaloes (n=500).

GIT Helminths Species	Number of Examined	Number of Positive	% age
	Fecal Samples	Fecal Samples	Infected
Toxocara vitulorum	500	83	16.6
Paramphistomum cervi	500	75	15
Fasciola hepatica	500	42	8.4
Oesophagostomum radiatum	500	16	3.2
Bunostomum phlebotomum	500	8	1.6
Cooperia spp.	500	8	1.6
Trichostrongylus spp.	500	4	0.8

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Table 2. Age-wise prevalence of different species of helminths in buffaloes (n=500).

Species of helminths	Calf	Adult	
Toxocara vitulorum	60/200; 30%	23/300; 7.67%	
Paramphistomum cervi	40/200; 20%	35/300;11.67%	
Fasciola hepatica	16/200; 8%	28/300; 9.33%	
Oesophagostomum radiatum	4/200; 2%	12/300; 4.00%	
Bunostomum phlebotomum	4/200; 2%	4/300; 1.33%	
<i>Cooperia</i> spp.	2/200; 1%	6/300; 2.00%	
Trichostrongylus spp.	1/200; 0.5%	3/300; 1.00%	

Table 3. Sex-wise prevalence of different species of helminths in buffaloes (n=500).

Species of helminth	Male	Female	
Toxocara vitulorum	25/125; 20%	58/375; 15.47%	
Paramphistomum cervi	30/125; 24%	45/375; 12%	
Fasciola hepatica	13/125; 10.4%	29/375; 7.73%	
Oesophagostomum radiatum	8/125; 6.4%	8/375; 2.13%	
Bunostomum phlebotomum	5/125; 4%	3/375; 0.8%	
Cooperia spp.	2/125; 1.6%	6/375; 1.6%	
Trichostrongylus spp.	0/125; 0%	4/375; 1.07%	

Table 4. Presence of various species of GIT helminths in buffalo.

Species of helminthes	Buffaloes
Nematodes	
Toxocara vitulorum	+
Oesophagostomum radiatum	+
Bunostomum phlebotomum	+
Cooperia spp.	+
Trichostrongylus spp.	+
Trematodes	
Fasciola hepatica	+
Paramphistomum cervi	+
Cestodes	-
Total Number of <i>Helminths</i> spp.	7

Age-wise animals	Calf	Adult	Overall prevalence
Buffaloes	159/200; 79.5%	141/300; 47.00%	300/500;60%
Sex-wise animal	Male	Female	Overall prevalence
Buffaloes	98/125; 78.4%	191/375; 50.93%	289/500; 57.8

Table 5. Age-wise and sex-wise prevalence of GIT helminths in buffalo (n=500).

spp., Fasciola hepatica and Paramphistomum cervi.

The prevalence of helminthes was higher in buffalo (Table 2) calves as compared to adult buffalo. A total of 12.4% buffalo (62/500) had mixed infection comprising 16% (32/200) in calves and 10% (30/300) in adult buffalo.

In Table 2 and 3 The prevalence of helminthes was higher in young animals compared with the older ones and the prevalence of helminthes was higher in males compared with the females.

In Table 4 a total of seven species of helminthes (five nematodes and two trematodes) were recorded.

In Table 5 the prevalence of helminthes was higher in young animals/ calves (79.5%) compared with the older ones (47%) while sex-wise the prevalence of helminthes was higher in males (78.4%) as compared with the females (50.93%).

DISCUSSION

Helminthiasis being one of the major problems affecting the productivity of buffaloes remains on top of the list. This severity depends on the prevalence, intensity of infection, presence of intermediate host, fauna and flora, frequency of infection, fecunditity and mal-management practices. In this cross sectional survey, the Agewise prevalence of helminthes was higher in young animals as compared to elder ones, and in sex-wise higher in males as compared with the females. In buffaloes, a total of seven species of helminthes including five nematodes, including Toxocara vitulorum, Oesophagostomum radiatum, Bunostomum phlebotomum, Cooperia spp., and Trichostrongylus spp. were found while two species of trematodes, i.e. Fasciola hepatica, Paramphistomum cervi were recorded. The most prevalent nematode recovered in this study area from buffaloes was T. vitulorum which was reported to be the most frequent occurring nematode in cattle and buffaloes by other scientists like El-Maukdad (1979), Iqbal et al. (1984), Mourad et al. (1985), Anwar et al. (1996) and Motahar et al. (2000).

The infection was higher in young animals as compared to older ones that may be attributed to lesser immunity because of fewer/ maiden exposure to various species of helminthes. It might be interesting that prevalence was higher in males as compared with females. Normally, females are assumed to be more infected due to stress of pregnancy and parturition due to stall feeding in females around the termination of pregnancy and thus lesser exposure to pasture contamination. Most of the researchers have observed higher rates of nematode burden in female hosts as compared with



Figure 1. Prevalence of different species of helminths in buffaloes.



Figure 2. Prevalence of different species of helminths in buffaloes.



Figure 3. Age-wise prevalence of different species of helminths in buffaloes.



Figure 4. Sex-wise prevalence of different species of helminths.

the males like Asanji and Williams, 1987; Pal and Qayyum, 1992; Iqbal *et al.*, 1984; Maqsood *et al.*, 1996; Komoin *et al.*, 1999; Valcarcel and Garcia Romero, 1999.

In contrast to the current results, Gulland and Fox (1992) reported that prevalence and intensity of infection (faecal egg counts) were higher in males than females, except during the lambing periods, and decreased with increase in age in both sexes. Effect of reproductive cycle has been reported to affect the worm burdens in animals, which has an important epidemiological significance as Lyons et al. (1987, 1992) reported a progressive increase in the egg per gram (EPG) and number of helminths in ewes during and after the parturition period. This phenomenon has been attributed to a variety of reasons like seasonal changes, host factors, activation of hypobiotic larvae, parturition stress, poor nutritional status, peri-parturient relaxation in immunity (PPRI) or spring rise phenomenon, hormonal changes around parturition, breed differences etc. etc. In many parts of the world, parturition of grazing animals is synchronized to occur with the favorable climate to pasture growth and also suitable for development and survival of free-living stages of most helminths (Wedderburn, 1970).

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