

POINT PREVALENCE OF GASTROINTESTINAL HELMINTHIASIS IN BUFFALOES (*Bubalus bubalis*) AT THE VICINITY OF JATOI, PUNJAB, PAKISTAN

Muhammad Asif Raza*, Hafiz Allah Bachaya¹, Muhammad Saleem Akhtar², Hafiz Muhammad Arshad², Saeed Murtaza², Muhammad Mazhar Ayaz², Muhammad Naeem³ and Abdul Basit²

*Animal Husbandry in Tropics and Subtropics, University of Kassel, Germany/ University of Agriculture, Faisalabad, Pakistan

¹Livestock & Dairy Development Department, Punjab, Pakistan

²Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan

³Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakariya University, Multan, Pakistan

*Corresponding author: asifrazarana@yahoo.com, asif-raza@icdd.uni-kassel.de

ABSTRACT: In the year 2009, a total of 500 faecal samples from buffaloes from different locations of Tehsil Muzaffar Garh were analyzed to confirm the presence of gastrointestinal parasitic infection. The recovered parasites were five nematodes and two trematodes. The nematodes were *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, respectively while sex wise prevalence was 78.4% and 50.93% in male and female buffalo, respectively. To know the dependence of helminth's prevalence on sex and age of animals, Chi-square statistical design was applied to data.

Key words: Buffaloes, Helminths, Prevalence, Jatoi, Pakistan

INTRODUCTION

Helminths are recognized as a major constraint to livestock production throughout the tropics and elsewhere [1, 2]. Helminth infections of water buffaloes are considered common in tropical and sub-tropical countries where they cause huge economic losses as a consequence of deaths of infected animals, reduced rates of weight gains and the condemnation of infected organs after slaughter [3]. Economic losses are caused by gastrointestinal parasitism are in a variety of ways: they cause losses through lowered fertility, reduced work capacity, involuntary culling, a reduction in food intake and lower weight gains, lower milk production, treatment costs, and mortality in heavily parasitized animals [4].

Prevalence of gastrointestinal helminthes in ruminants has been reported from 25 to 92% in different areas of Pakistan at different times [18, 24, 28, 29, 35, 36]. Facioliasis, parasitic gastro enteritis, hydatidosis, coccidiosis, ectoparasitism, theileriosis and babesiosis are the major parasitic problems of ruminants in Pakistan in the order of priority [5]. There are a number of factors that influence the prevalence of helminths. These include age [6], sex [7], breed [8, 9], worm population [10], weather condition [7,11] and husbandry or management practices [12].

Some helminths infecting buffaloes primarily are also transmissible (directly or indirectly) to humans where they can cause significant clinical disease, such as schistosomiasis, cystic echinococcosis, and fasciolosis [3, 13]. Considering the health implications and the economic potential of water buffaloes (*Bubalus bubalis*), the issue of investigating parasitic infections in buffaloes is of considerable relevance [14, 15].

MATERIAL AND METHODS

The present study was conducted from January to June 2009 to determine the point prevalence of gastrointestinal helminthiasis in the buffalo under field conditions at the vicinity of Tehsil Muzaffar Garh of District Muzaffar Garh, Southern Punjab, Pakistan.

Study area

From January to June 2009, faecal samples of total 500 buffaloes were brought to District Diagnostic Lab. Muzaffargarh for analyses. District Muzaffar Garh, positioned between latitude 288570 to 308460 N and longitude 708300 to 718470 E, having an area of 8249 km² which lies in the form of strip between the rivers Chenab and Indus, which pass along the eastern and western boundaries, respectively, of the district and form a triangle at Alipur tehsil of the district.

Sample collection

One thousand faecal samples of buffaloes were randomly collected in sterile polythene bags directly from rectum of each animal [16]. These faecal samples were brought to District Diagnostic Laboratory Muzaffar Garh and Department of Pathobiology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan for identification of eggs/larvae or adult helminthes.

Sample analyses

Faecal samples were examined for helminthes eggs/larvae by using direct and indirect techniques [16] and for identification of certain nematodes, copro-culture were performed to obtain larval stage. Both eggs and larvae from copro-culture were identified by using standard techniques as described by MAFF [32] and Soulsby, E. J. L [37]. 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 concentration techniques, i.e. floatation and sedimentation.

For floatation technique, five grams of feces was mixed in 30-50 ml of water and strained through a sieve to remove the coarse material. The mixture was allowed to sediment for half an hour. The supernatant was poured off and sediment was mixed in a saturated solution of common salt. 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 developed as micro-floatation technique [16].

For sedimentation technique, five grams of faeces was mixed in 30-50 ml of water and strained through a sieve to remove the coarse 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 was taken from sediment with Pasteur's pipette on slide and was examined under microscope at 10 X for the presence of helminthes eggs.

Copro-culture

Faecal cultures provide an environment suitable for hatching and development of helminthes eggs. Feces found positive for nematode eggs but confusing for exact identification were broken up finely, using either a large pestle and mortar or spatula and were placed in a glass jar or petri-dish which was closed and incubated at a temperature of about 26°C for 7 days. After incubation, samples were examined for the presence of larvae. Larvae were identified with the help of keys given by [32].

Statistical analyses

Data on the prevalence of helminthiasis was analyzed using Chi-square statistical design on the basis of sex and age.

Graphical representation of data was also done.

RESULTS

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

Table I. Prevalence of different species of helminths in buffaloes

Species of helminth	Number of Faecal Samples Examined	Number of Faecal Samples Positive	Per cent Infected % age
<i>Toxocara vitulorum</i>	500	83	16.6
<i>Paramphistomum cervi</i>	500	75	15
<i>Fasciola hepatica</i>	500	42	8.4
<i>Oesophagostomum radiatum</i>	500	16	3.2
<i>Bunostomum phlebotomum</i>	500	8	1.6
<i>Cooperia</i> spp.	500	8	1.6
<i>Trichostrongylus</i> spp.	500	4	0.8

The prevalence of helminthes was higher in buffalo (Table-II) 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.

Table II. Age-wise prevalence of different species of helminths in buffaloes in Tehsil Muzaffar Garh, District Muzaffar Garh

Species of helminth	Calf	Adult
<i>Toxocara vitulorum</i>	60/200; 30%	23/300; 7.67%
<i>Paramphistomum cervi</i>	40/200; 20%	35/300; 11.67%
<i>Fasciola hepatica</i>	16/200; 8%	28/300; 9.33%
<i>Oesophagostomum radiatum</i>	4/200; 2%	12/300; 4.00%
<i>Bunostomum phlebotomum</i>	4/50; 2%	4/300; 1.33%
<i>Cooperia</i> spp.	2/200; 1%	6/300; 2.00%
<i>Trichostrongylus</i> spp.	1/200; 0.5%	3/300; 1.00%
Mixed infection	32/200; 16%	30/300; 10.00%
Overall prevalence	159/200; 79.5%	141/300; 47.00%

Table III. Sex-wise prevalence of different species of helminths in buffaloes in Tehsil Muzaffar Garh, District Muzaffar Garh

Species of helminth	Male	Female
<i>Toxocara vitulorum</i>	25/125; 20%	58/375; 15.47
<i>Paramphistomum cervi</i>	30/125; 24%	45/375; 12
<i>Fasciola hepatica</i>	13/125; 10.4%	29/375; 7.73%
<i>Oesophagostomum radiatum</i>	8/125; 6.4%	8/375; 2.13%
<i>Bunostomum phlebotomum</i>	5/125; 4%	3/375; 0.8%
<i>Cooperia</i> spp.	2/50; 1.6%	6/375; 1.6%
<i>Trichostrongylus</i> spp.	0/125; 0%	4/375; 1.07%
Mixed infection	15/125; 12%	38/375; 10.13%
Overall prevalence	98/125; 78.4%	191/375; 50.93%

In Table II and III, The prevalence of helminths was higher in young animals compared with the older ones and the prevalence of helminths was higher in males compared with the females.

Table IV. Prevalence of different species of helminths in animals in Tehsil Muzaffar Garh, District Muzaffar Garh

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

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

Table V. Age-wise and sex-wise prevalence of different species of helminths in buffalo in Tehsil Muzaffar Garh, District Muzaffar Garh

Species of animal	Calf	Adult	Overall prevalence
Buffaloes	159/200; 79.5%	141/300; 47.00%	300/500; 60%
Species of animal	Male	Female	Overall prevalence
Buffaloes	98/125; 78.4%	191/375; 50.93%	289/500; 57.8

In Table V, 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 is one of the major problems which affect the productivity of buffaloes. Losses caused by helminthes invariably depend on the prevalence, nature and intensity of infection and the management practices. In this investigation, the prevalence of helminthes was higher in young animals compared with the older ones, and higher in males compared with the females in this study. In buffaloes, a total of seven species of helminthes including five nematodes, i.e. *Toxocara vitulorum*, *Oesophagostomum radiatum*, *Bunostomum phlebotomum*, *Cooperia* spp., *Trichostrongylus* spp.; two trematodes, i.e. *Fasciola hepatica*, *Paramphistomum cervi* were recorded. The most prevalent nematode recovered in this study from buffaloes were *T. vitulorum*. *T. vitulorum* was also reported to be the most frequently occurring nematode in cattle and buffaloes [18, 25, 26, 33, 34].

The higher infection in young animals than that in older ones may be attributed to lesser resistance because of lesser exposure to different species of helminthes compared with the

older animals. It was, however, interesting to note that prevalence was higher in males compared with females. Normally, females are assumed to be more infected due to stress of pregnancy and parturition. This may be due to the practice of stall feeding females around pregnancy and thus lesser exposure to pasture contamination. Most of the researchers have observed higher rates of nematode infection/worm burden in female hosts compared with the males [7, 17, 18, 19, 20, 21]. Higher prevalence of nematode parasites in females compared with males may be because of lowered resistance of female animals due to their reproductive events and insufficient/unbalanced diet against higher needs. In contrast to the current results, [27] Gullrand 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 age in both sexes. Effect of reproductive cycle has been reported to affect the worm burdens in animals, which has an important epidemiological significance. For example, Lyons *et al.* (1987, 1992) and reported a progressive increase in the egg per gram 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), hormonal changes around parturition, breed differences etc. etc. In many parts of the world, parturition of grazing animals is synchronized to occur with the climate favorable to pasture growth and also suitable for development and survival of free-living stages of most helminths [22].

REFERENCES

- Ibrahim, M.A., N. Nwude, R.A. Ogunsusi and Y.O. Aliu. Screening West African plants for anthelmintic activity. *ILCA Bull.*, **17**: 19-23, 1984.
- Waller, P. J. Anthelmintic resistance. *Vet. Parasitol.*, **72**: 391-412, 1987.
- Liu Y, Li F, Liu W, Dai RS, Tan YM, He DS, Lin RQ, Zhu XQ. Prevalence of helminths in water buffaloes in Hunan province, China. *Trop. Anim. Health Prod.*, **41**: 543-546, 2009.
- Lebbie S.H.B., Rey B. and Irungu E.K.. 1994. Small ruminant research and development in Africa, Proceedings of the Second Biennial Conference of the African Small Ruminant Research Network, ILCA (1994), pp. 1-5.
- Iqbal, Z., M.N. Khan and A. Qudoos, 2002. Parasitic research on domesticated animals of Pakistan. University Grants Commission, Islamabad-Pakistan.
- Mckenna, P.B. The diagnostic value and interpretation of faecal egg counts in sheep. *New Zealand Vet. J.*, **29**: 129-132, 1981.
- Asanji, M. F. and O. Williams. Variables effecting the population dynamics of gastrointestinal helminth parasite of small farm ruminant in Sierra Leone. *Bull Anim. Hlth. Prod. Africa*, **35**: 308-313, 1987.
- Miller, J. E., M. Bahirathan, S. L. Lemarie, F. G. Hembry, M. T. Kearney and S. R. Barras. Epidemiology of gastrointestinal nematode parasitism in Suffolk and Gulf Coast Native sheep with special emphasis on relative susceptibility to *Haemonchus contortus* infection. *Vet. Parasitol.*, **74**: 55-74, 1998.
- Mirza, M.A and A. Razzak. Internal parasitism in sheep and goats under extensive grazing system. *Pak. Vet. J.*, **18**: 53-54, 1998.
- Ankers, P., S. Fofana and A.E. Biaye. Epidemiology of helminths of cattle, sheep and goats in Maritime Guinea, Guinea. *Revue-d'Elevage-et-de-Medecine-Veterinaire-des-Pays-Tropicaux*, **50**: 111-116, 1997.
- Mohiuddin, A., M.M. Khan, F.A. Mughal and M.A. Sheikh. Taxonomy, incidence and seasonal variations of helminth parasites of sheep and goats of Sind. *Pak. J. Zool.*, **16**: 25-30, 1984.
- Tan, L.X., X.H. Gong, R.F. Tan, Y.L. Ni, Wang and Z.M. Guo. The efficacy of an albendazole-mediated blocks in controlling sheep nematodes in Xinjiang province. North West China. *Vet. Res. Comm.*, **20**: 427-435, 1996.
- Cringoli G, Rinaldi L, Musella V, Veneziano V, Maurelli MP, Di Pietro F, Frisiello M, Di Pietro S. Geo-referencing livestock farms as tool for studying cystic echinococcosis epidemiology in cattle and water buffaloes from southern Italy. *Geospat Health*, **2**: 105-111, 2007.
- Rinaldi L, Musella V, Condoleo R, Saralli G, Veneziano V, Bruni G, Condoleo RU, Cringoli G. Giardia and Cryptosporidium in water buffaloes (*Bubalus bubalis*). *Parasitol. Res.*, **100**: 1113-1118, 2007.
- Veneziano V, Santaniello M, Carbone S, Pennacchio S, Morgoglione ME, Schioppi M, Condoleo R, Cringoli G. Lice (*Haematopinus tuberculatus*) in water buffalo farms from central Italy. *IJAS*, **6**: 926-927, 2007.
- Ayaz, M.M. 2010. The procedures in Veterinary Protozoology. 1st ed. BBS life sciences pub. Multan. Pakistan
- Pal, R.A. and M. Qayyum. Breed, age and sex-wise distribution of gastro-intestinal helminths of sheep and goats in and around Rawalpindi region. *Pak. Vet. J.*, **12**: 60-63, 1992.
- Iqbal, N. C., M. S. Durrani and A. Tariq. The incidence of gastrointestinal parasite in buffalo and cattle of Azad Kashmir. *Pak. Vet. J.*, **4**: 60-61, 1984.
- Maqsood, M., Z. Iqbal and A.H. Chaudhry. Prevalence and intensity of haemonchosis with reference to breed, sex and age of sheep and goats. *Pak. Vet. J.*, **16**: 41-43, 1996.
- Komoin, Oka. C., J. Zinsstag, V.S. Pandey, F. Fofana and A.N. Depo. Epidemiology of parasites of sheep in the southern forest zone of Coted'Ivoire. *Revue-d'Elevage-et-de-Medecine-Veterinaire-des-Pays-Tropicaux*, **52**: 39-46, 1999.
- Valcarcel, F. and C. Garcia Romero. Prevalence and seasonal pattern of caprine trichostrongyles in a dry area of central Spain. *J. Vet. Med. Series B.*, **46**: 673-681, 1999.
- Wedderburn, J. F. Ostertagiasis in adult cattle: a clinical report of outbreak in the field. *New Zealand Vet. J.*, **18**: 168-170, 1970.
- Ali, S., M. Q. Khan, M. Qayyum and M. F. U. Khan. Prevalence of gastrointestinal parasites in sheep and goats maintained at NARC, Islamabad, Pakistan. *Pak. Vet. J.*, **20**: 157-158, 2000.
- Al-Shaibani, I. R. M., M. S. Phulan, A. Arijo and T. A. Qureshi. Epidemiology of ovine gastro-intestinal nematodes in Hyderabad district, Pakistan. *Pak. Vet. J.*, **28**: 125-130, 2008.
- Anwar, A. H., C. S. Hayat and M. I. Amir. Prevalence of gastro intestinal helminthiasis and comparative efficacy of anthelmintics in parasitized buffalo calves. *Pak. Vet. J.* **16**: 160-163, 1996.
- El-Moukdad, A. R. Helminths of cattle in Syria. *Angewandte Parasitologie*, **20**: 11-16, 1979.
- Gulland, F.M.D and M. Fox. Epidemiology of nematode infections of Soay sheep (*Ovis aries* L.) on St Kilda. *Parasitol.*, **105**: 481-492, , 1992.
- Ijaz, M., M. S. Khan, M. Avais, K. Ashraf, M. M. Ali and Saima. Infection rate and chemotherapy of various helminths in goats in and around Lahore. *Pak. Vet. J.*, **28**: 167-170, 2008.
- Kakar, M. N. and J. K. Kakarsulemankhel. Prevalence of endo (trematodes) and ecto-parasites in cows and buffaloes of Quetta, Pakistan. *Pak. Vet. J.*, **28**: 34-36, 2008.

30. Lyons, E. T., J. H. Drudge and S.C. Tolliver. Epizootology of internal parasites in lambs and ewes during periparturient period in Kentucky in 1986. *Proc. Helminthol. Soc. Washington*, **54**: 233-236, 1987.
31. Lyons, E. T., S.C. Tolliver, J. H. Drudge and S. Stampfer. Internal parasites in a small flock of lambs and ewes during the periparturient in 1987 in Kentucky. *Trans. Ky. Acad.*, **53**: 15-18, 1992.
32. MAFF, Parasitological laboratory techniques, *Technical Bulletin* No. 18. Ministry of Agriculture Fisheries and Food Manual of Veterinary, Her Majesty's Stationary Office, London. 1979.
33. Motahar, M. H. Examination of gastrointestinal helminth in livestock grazing in grassland of Bangladesh. *Korean J. Parasitology*, **38**: 187-190, 2000.
34. Mourad, M. I., I. S. A. Abdullah and T. E. Allowy. Comparative study on the gastrointestinal parasitism of cattle & buffalo with special reference of haematological changes at Assiut Governorate. *Assiut Vet. Med. J.*, **15**: 163-166, 1985.
35. Raza M.A., S Murtaza, H. A. Bachaya, A Qayyum and M. A. Zaman. Point prevalence of *Toxocara vitulorum* in large ruminants slaughtered at Multan abattoir. *Pak. Vet. J.*, **30**: 242-244, 2010.
36. Raza, M. A., Z. Iqbal, A. Jabbar and M. Yaseen. Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakiatan. *J. Helminthol.*, **81**: 323-328, 2007.
37. Soulsby, E. J. L., *Helminths, Arthropods and Protozoa of domestic animals*. 7th Ed. Baillier Tindall and Cassel Ltd. London 1982..