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Prevalence of *Giardia lamblia* and Gastrointestinal Parasites in Ruminants

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Abstract: The present study was conducted to determine the prevalence of *Giardia Lamblia* and Gastrointestinal parasites in Ruminants. One hundred and fifty fecal samples were collected randomly from the sheep and goats, cattle and buffaloes in different location of Islamabad (villages, dairy farms), Chitral and Peshawar. The samples were screened for the presence of Gastrointestinal parasites using microscopic techniques, (simple test tube flotation and sedimentation technique), centrifugation technique (formalin ethyl-acetate sedimentation technique) and fecal culture. *Eimeria* species were detected in 31 samples (20.66%), *Fasciola* in 22 samples (14.66%), *Haemonchus* in 19 samples (12.66%), *Ostertagia* in 12 samples (8%), *Trichostrongylus* in 9 samples (6%) and Giardia species in 24 samples (16%) respectively. These results demonstrate that environmentally resistant cysts or eggs could be widespread on the farms examined and thus an effective hygienic management system is needed to prevent them from serving as the source of infection for human beings.

Key words: Cysts · *Giardia lamblia* · Gastrointestinal Parasites · Ruminants

INTRODUCTION

Gastrointestinal [GI] parasites cause'sparasitisms in ruminants are responsible for significant production losses in livestock worldwide particularly under tropical and subtropical climates [1, 2]. These parasites adversely affect the health status of animals and cause enormous economic losses to the livestock industry. Gastrointestinal parasites not only affect the health but also affect the productive and reproductive performance of ruminants [3, 4]. GI parasites populate in the gastro-intestinal tract of humans and other animals [5]. Unlike predators, parasites are generally much smaller than their host; both are special cases of consumer-resource interactions [6]. Parasites show a high degree of specialization and reproduce at a faster rate than their hosts. Classic examples of parasitism include interactions between vertebrate hosts and diverse animals such as tapeworms, flukes, the *Plasmodium* species and fleas. The major groups of parasites include protozoans (organisms having only one cell) and parasitic worms (helminths). Of these, protozoans, including cryptosporidium, microsporidia and isospora, are most common in HIV-infected persons. Each of these parasites can infect the digestive tract and sometimes two or more can cause infection at the same time [7].

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Giardiasis is a diarrheal infection in the small intestine of animal and humans, which is caused by a single-celled organism known as *Giardia duodenalis*. Giardia duodenalis is a protozoan parasite which is found in the small intestine of vertebrates including mammals [8]. Giardia duodenalis cause intestinal diseases in all ruminants and mostly occur in calves of ruminants. The species of *G. duodenalis* (*G. intestinalis* and *G. lamblia*) has been identified in cats [9]. Most infections of these protozoa are subclinical or show the transient softening of the stool in the early stages of the infection, although diarrhea may be acute, chronic, or intermittent in dogs and cats. Clinical signs are mostly identified in the younger animals from multi-cat households [10-13]. The transmission of this infection is due to the ingestion of feces or fecal-contaminated water or food. The life cycle of giardiasis consist of two stages. Trophozoites are the active motile form of the infection which moves towards the colon of the intestine where they produce a cyst form. The cysts are extremely hard and can be survived for long time in water. The parasite has a one to two week incubation period. [14-17].

To ensure the health and well-being of pet dogs and cats, coprologic examinations for parasite eggs, oocysts and cysts are an important part of the daily routine for most veterinary practices. Although a fecal examination is considered a routine procedure in many clinics, it has been our experience that often little thought is given to performing the procedure correctly [18]. Many different procedures and techniques are used, each with its own advantages and limitations. For example, direct fecal smears are useful for detecting motile protozoa, whereas sedimentation examinations are more suitable for recovering heavy (e.g., *Physaloptera*spp) or operculated (e.g., fluke) eggs that do not float well because of the hypertonic effects exerted by the flotation solution. However, the methods used most frequently to recover parasite eggs, oocysts and cysts are flotation techniques, which rely on the differences in the specific gravity (SG) of the egg(s), fecal debris and flotation solution [18]. For parasite eggs to float, the SG of the flotation solution must be greater than that of the eggs. Flotation solutions are made by adding a measured amount of salt or sugar to a specific amount of water to produce a solution with the desired SG; such solutions are effective, easy to make or commercially available and relatively inexpensive. It is important to ensure that the flotation solution used has the proper SG, which is best accomplished by using a hydrometer calibrated to measure in the desired range. Hydrometers used to measure urine SG do not cover the required SG range needed for fecal examinations [18].

Giardiasis in particular is a diagnostic dilemma. We agree that Giardia is one of the most commonly misdiagnosed, under-diagnosed and over diagnosed parasites. Many veterinary practices find it difficult to diagnose giardiasis using fecal examinations. Many pseudo parasites such as yeasts plant remnants and debris have been mistaken for these tiny organisms. Identification of Giardia cysts is further compromised because microscopes used in private practice are often not equipped with micrometers that can allow measurement of cysts that are as small as 8 to 12 × 7 to 10 µm [18]. Giardia cysts are particularly difficult to recover and identify. The cysts are small and fragile and infected animals shed the cysts intermittently. Several studies have demonstrated that recovery of Giardia cysts can best be accomplished using a 1.18-SG ZnSO4 centrifugation technique, [19-22].

MATERIALS AND METHODS

Collection of Samples: Larvae were recovered from fecal samples collected from different animals like cattle, buffalo, sheep and goat. These samples were brought to National Veterinary Laboratories (NVL), Islamabad Pakistan.

Simple Test Tube Flotation: Approximately 3 g of feaces was measured with a precalibrated teaspoon and was put into Container. After 20 min the cover slip was taken off from the tube, together with the drop of fluid adhered to it and immediately the cover slip was placed on a microscope slide [23].

Sedimentation Technique: 3 g of feaces was measured into Container 1.Then 40-50 ml of tap water was added into Container.The sediment was strained by adding one drop of methylene blue.At last the sediment was transferred to a microslide covered with a coverslip [23].

Formalin Ethyl-Acetate Sedimentation Technique: First of all feaces were preserved in 10% formalin. Then a piece of feaces was passed through a sieve into about 9ml of water and then solution was poured into 15ml centrifuge tube. 3ml ethyl-acetate was added and the tube was plugged with a rubber stopper. Then the tube was

vigorously shacked and was centrifuge it at 1500 rpm for 10min. The supernatant was poured off very carefully to leave the pellet at the bottom of the tube. Some of the sediment was transferred from the bottom of the tube to a slide and was examined under the microscope. The cyst and trophozites of Giadia species was checked out [23].

Preparation of Fecal Cultures: Many nematode eggs are alike and species such as *Haemonchus, Mecistocirrus, Ostertagia, Trichstrongylus, Cooperia, Bunostomum* and *Oesophagostomum* cannot be clearly differentiated from the eggs in faecal samples. For these parasites, differentiation can be achieved by the use of faecal cultures [23]. The identification of parasite species present is an important component of initial surveys and of the investigation of clinical disease caused by gastrointestinal nematodes [25].

RESULTS

In the present study 150 fecal samples were collected from different location for the diagnosing of different type of gastrointestinal parasites and specially Giardia species (*G. lamblia*). These samples were randomly collected from small ruminants like sheep and goats and large ruminants like cattle and buffaloes. These samples were carried to the NVL (National Veterinary Laboratories) for the screening test for the detection of various gastrointestinal parasites. Out of 150 samples 22 samples were found positive for *Fasciola*, 19 for *Haemonchus*, 12 for *Oestertagia* and 09 samples were positive for *Trichostrongylus* species. Gastrointestinal protozoan parsites, *Eimeria* and Giardia were found in 31 and 24 of the total samples respectively as shown in Table 1.

Table 1: Incidence of parasitic species in the fecal samples (n=150).

Parasites	Total sample positive	Percentage positive (%)
Eimeria	31	20.66
Fasciola	22	14.66
Haemonchus	19	12.66
Ostertagia	12	8
Trichostrongylus	09	6
Giardia	24	16

The parasites were identified in fecal samples by using different techniques. Ethyl-acetate sedimentation was found to be the most suitable technique used for the detection of these parasites. Simple test tube flotation, sedimentation and Ethyl-acetate sedimentation techniques were used for the detection of *Fasciola, Haemonchus, Oestertagia,Trichostrongylus* and also for the other gastrointestinal parasites like *Eimeria* and Giardia. Majority of the Giardia species were detected by the Formalin ethyl-acetate sedimentation technique (Table 2). After identification positive samples were cultured to further study the morphological characteristics for conformation. Giardia egg, Eimeria ocyst andTrichostrongylus ocyst were identified by using 40x magnification in microscope as shown in Figure 1.

The field study was carried out at different locations in Pakistan. Samples were collected from Chitral, dairy farm of NARC Islamabad, Villages of Islamabad and

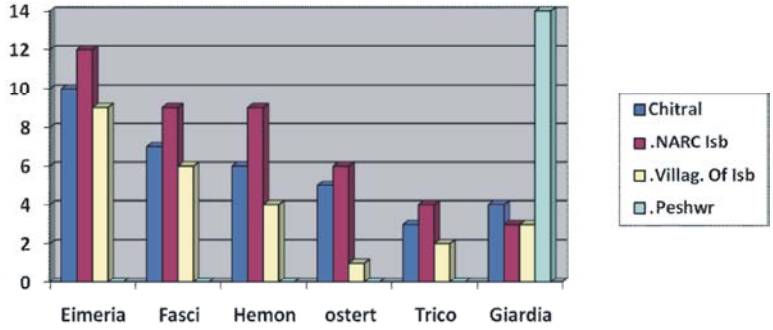

Fig. 1: Graphical representation of fecal sample collected from Chitral, Dairy farm of NARC Islamabad, Villages of Islamabad and Peshawar.

Table 2: Parasites identified in fecal samples using different techniques.

Techniques	Eimeria	Fasciola	Haemonchus	Ostertagia	Trichostr-ongylus	Giardia species
Simple test tube flotation	8	9	7	2	1	0
Sedimentation technique	12	11	8	5	4	15
Ethyl-acetate Sedimentation	15	12	17	9	6	21
Total	35	32	32	16	11	36

Table 3: Number of parasites detected in fecal samples brought from different locations

	Location			
Parasites	Chitral	Dairy farm of NARC Islamabad	Villages of Islamabad	Peshawar
Eimeria	10	12	9	0
Fasciola	7	9	6	0
Haemonchus	6	9	4	0
Ostertagia	5	6	1	0
Trichostrongylus	3	4	2	0
Giardia species	4	3	3	14

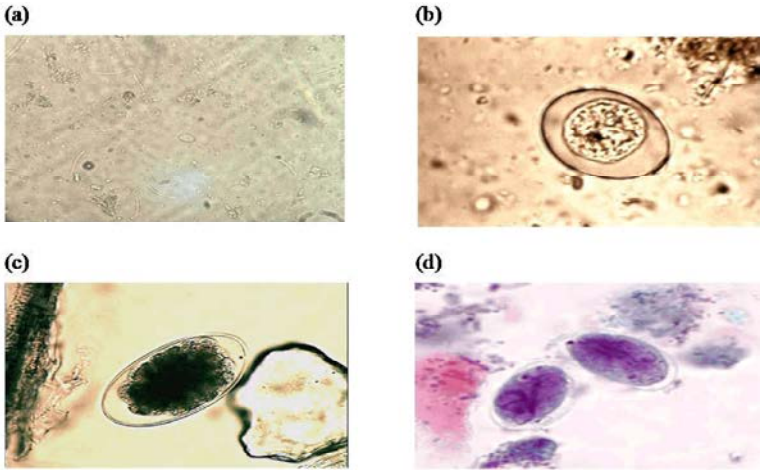

Fig. 2: Microscopic examination of identified Giardia egg (a); Eimeria ocyst (b);Trichostrongylus ocyst (c) and Giardia egg (d) by using 40x magnification.

Peshawar as shown in Figure 2. Among the identified parasites Giardia was prevalent in the villages of Peshawar, followed by *Eimeria* which was found in the dairy farm of NARC in Islamabad. The villages of Islamabad are relatively less affected by the studied parasites.

The present investigation revealed that the internal parasitic fauna of livestock was composed of a number of helminthes and intestinal protozoa. Most important helminthes that were identified from the fecal samples were *Hemonchus, Fasciola, Ostertagia* and *Trichostrongylus* species. While there was high incidence of gastrointestinal protozoan parasites like *Eimeria*, Giardia and Cryptosporidium species in the fecal samples collected from different locations (Table 3). Their incidence differed from one region to another and according to the species of the animals. These infestations resulted in both clinical and subclinical forms of parasitism, which induced direct and indirect losses. The direct losses were due to acute illness and death. However, indirect losses can be observed which causes a decrease in the productivity potential of livestock including milk, meat and wool production.

These economic losses due to gastrointestinal Helminthes and Protozoan parasites can be overcome by use of regular deforming of the livestock according to prescribed schedule. Early detection of these parasitic infections and treatment can also help in reducing losses in the terms of productivity.

DISCUSSION

Pakistan has a large livestock population, which is well adapted to local conditions and some of the best tropical breeds. Between the 1955 and 1996, there were an estimated 20.7 million buffaloes, 17.9 million cattle, 30.5 million sheep, 47.6 million goats, 1.2 million camels and 380 million poultry. Buffaloes are kept mainly in the northern and southern irrigated plains and cattle are raised throughout the country. Pakistan has population of 27.3 million of buffaloes, which play an important role in the national economy of the country and are the major source of meat and milk. Out of total milk (38.38 million tons) produced in Pakistan, 64% comes from buffaloes [25].

There are some factors, which affect the production performance of buffaloes. Among these diseases caused by different viruses, fungi, bacteria and parasites which cause great economic losses in terms of mortality and decreased milk production. It is an established fact that parasitic diseases present a far greater threat to the livestock than visible outbreaks of the diseases. These dormant infestations adversely affect the whole flock or herd leading to retarded growth rate, lower milk yield, milk quality, causing unthriftiness, poor furnishing and predisposing for bacterial and viral diseases due to stress and body damage. Economic losses may be obvious like death, wasting condemnation of parts used as human food and hidden losses like reduced live weight gain, poor feed conversion, reduced lactation and poor fleece etc. Internal parasites constantly affect the production and health of livestock. Nematode infestation lowers the resistance of animals and predisposes them to secondary infestations [26]. 470 million Rupees annual economic losses estimated were caused due to parasitic diseases of animals in Pakistan [27].

In this study different type of gastrointestinal parasites and specially Giardia species (*G. lamblia*) were detected in small ruminants (sheep and goats) and large ruminants (cattle and buffaloes) in dairy farm of Villages of Chitral, Islamabad, Peshawar and dairy farm of NARC Islamabad, Pakistan. Among the identified parasites Giardia was prevalent in the villages of Peshawar. Giardia *is a* genus of ubiquitousintestinal flagellates and well-known enteric parasite affecting a wide range of vertebrate hosts including humans and a range of domestic and wild mammals. The prevalence of infection with Giardia was high in the fecal samples of tested ruminants in the selected farms. Giardiasis is an intestinal infection and is a common cause of morbidity in humans. It is caused by a flagellate parasite known as *Giardia lamblia* (*G. Lamblia*) which is a one celled microscopic organism [28-30]. The organism produces environmentally resistant cysts which are voided in the faeces and transmitted directly, or via water or food, to another host, with infection resulting from ingestion. It is one of the leading causes of diarrhoea in children, as distinct from *Entamoeba histolytica* which causes diarrhea especially in adults [31].

Giardiasis is an emerging problem and often considered to be a disease of developing countries due to poor sanitation and lack of portable water supply. Water is increasingly recognized as an important vehicle for the transmission of Giardia [32, 33]. The disease is worldwide in distribution, to the extent that even in developed nations where portable water could be contaminated with small amounts of sewage particularly if septic systems are built too close to water supply [34, 35]. The transmission of the cyst of *G. Lamblia* could occur from person to person or by feco-oral route, but commonly it is by contaminated drinking water and food.

REFERENCES

1. Gill, H.S. and L.F. LeJambre, 1996. Preface- Novel approaches to the control of helminth parasites of livestock. International Journal of Parasitology, 26: 797-798.
2. Waller, P.J., 1997. Nematode parasite control of livestock in the tropics/subtropics: the need for novel approaches. International Journal of Parasitology, 27: 1193-1201.
3. Waller, P.J., 1999. International approaches to the concept of integrated control of nematodes parasites of livestock. International Journal of Parasitology, 29: 155-164.
4. Hayat, C.S., B. Hayat, M. Ashfaque and K. Muhammad, 1984. Bottle jaw in Berberi (Teddy) goat. Pakistan Veterinary Journal, 4: 183.
5. Loukopoulos, P., A. Komnenou, E. Papadopoulos and V. Psychas, 2007. Lethal Ozolaimusmegatyphlon infection in a green iguana (Iguana iguanarhinolopa). Journal of Zoo and Wildlife Medicine, 38: 131-134.
6. Getz, W., 2011. Biomass transformation webs provide a unified approach to consumer-resource modelling. Ecology Letters, doi:10.1111/j., 1461-0248,2010,01566.,x.
7. Bush, A.O., J.C. Fernández., G.W. Esch and J.R. Seed, 2001. Parasitism: the diversity and ecology of animal parasites. Cambridge Univ. Press.
8. Filippich, L.J., P.A. McDonnell, E. Munoz and J.A. Upcroft, 1998. Giardia infection in budgerigars. Australian Veterinary Journal, 76(4): 246-9.
9. Swan, J.M. and R.C. Thompson, 1986. The prevalence of Giardia in dogs and cats in Perth, Western Australia. Australian Veterinary Journal, 63(4): 110-2.
10. Adam, R.D., 2001. Biology of Giardia Lamblia. Clinical Microbiology Reviews, 14(3): 447-75.
11. Ponce-Macotela, M., G.E. Peralta-Abarca and M.N. Martínez-Gordillo, 2005. Giardia intestinalis and other zoonotic parasites: Prevalence in adult dogs from the southern part of Mexico City. Journal of the American Veterinary Medical Association, 131(1-2): 1-4.

12. Cutting, W.A.M., 1991. Diarrhoea diseases In: Stanfield, P. Brueton, M. Chan, M. Parkin, M and Waterston, T (eds). Diseases of Children in the subtropics and Tropics. Edward Arnold, 4th ed., pp: 455-495.
13. Palmer, C.S., R.C. Thompson, R.J. Traub, R. Rees and I.D. Robertson, 2008. National study of the gastrointestinal parasites of dogs and cats in Australia. Veterinary Parasitology, 151(2-4): 181-90.
14. Spain, C.V., J.M. Scarlett, S.E. Wade and P. McDonough, 2001. Prevalence of enteric zoonotic agents in cats less than 1 year old in Central New York StateJournal of Veterinary Internal Medicine, 15(1): 33-8.
15. McGlade, T.R., I.D. Robertson, A.D. Elliot and R.C. Thompson, 2003. A. High Prevalence of Giardia detected in cats by PCR. Veterinary Parasitology, 110(3-4): 197-205.
16. Robben, S.R., W.E. le Nobel, D. Dupfer, W.H. Hendrikx, J.H. Boersema, F. Fransen and M.E. Eysker, 2004. Infections with helminths and/or protozoa in cats in animal shelters in the Netherlands. Tijdschr Diergeneeskd, 129(1): 2-6.
17. De Santis-Kerr, A.C., M. Raghavan, N.W. Glickman, R.J. Caldanaro, G.E. Moore, H.B. Lewis, P.M. Schantz and L.T. Glickman, 2006. Prevalence and risk factors for Giardia and coccidia species of pet cats in 2003-2004. J. Fel. Med. Surg., 8(5): 292-301.
18. Dryden, M.W., P.A. Payne, R.K. Ridley and V.E. Smith, 2006. Gastrointestinal Parasites: The Practice Guide to Accurate Diagnosis and Treatment. Compendium on Continuing Education for the Practicing Veterinarian, 28: 8(A).
19. Zimmer, J.F. and D.B. Burrington, 1986. Comparison of four techniques of fecal examination for detecting canine giardiasis, Journal of the American Veterinary Medical Association, 22: 161-167.
20. Payne, P., M. Dryden, R. Ridley, *et al*., 2002. Evaluation of the efficacy of DrontalÔ Plus and GiardiaVaxÔ to eliminate cyst shedding in dogs naturally infected with Giardia spp. Journal of the American Veterinary Medical Association, 220(3): 330-333.
21. Barr, S.C., D.D. Bowman and N.E. Hollis, 1992. Evaluation of two procedures for diagnosis of giardiasis in dogs. American. Journal of Veterinary Research, 53: 2028-2031.
22. Zajac, A.M., J. Johnson and S.E. King, 2002. Evaluation of the importance of centrifugation as a component of zinc sulfate fecal flotation examinations, Journal of the American Veterinary Medical Association, 38: 221-224.
23. Roepstorff, A. and P. Nanson, 1998. Epidemiology, Diagnosis and Control of HelminthParasites of Swine: FAO Animal Health Manual, pp: 3.
24. Hansen J. and B. Perry, 1990. The Epidemiology, Diagnosis and Control of Gastro-lntestinal Parasites of Ruminants in Africa. A Handbook. ILRAD (International Laboratory for Research on Animal Diseases), Nairobi, Kenya, pp: 121.
25. Anonymous, 2008. Economic survey of Pakistan. Govt. of Pakistan, Economic advisor wing, Finance division, Islamabad.
26. Solusby, E.J.L., 1982. Helminths, Arthropodes and protozoa of domestic animals. 7th edi. Ball. Ten. Lon., pp: 12-82.
27. Durani, M.A., 1965. Food losses due to animal parasites, Seminar on food production and consumption, west Pakistan Agricultural University, Layallpur (Faisalabad).
28. Yakubu, A.M., 2007. Disorders of the intestinal tract. In Azubuike, J.C. and Nkanginieme, K.E.O., (eds). Paediatrics and child Health in Tropical Region. Owerri, Africa Educational Services 2nd Ed., pp: 268-282.
29. Aucott, J., 1996. Giardiasis and other protozoal Diseases, In: Behrman, RE; Kliegman, RM and Jenson, HR (eds). Nelson Textbook of Paediatrics. Philadelphia. WB Saunders Company, 15th ed., pp: 907-971.
30. Gupte, S., 2001. Pediatric parasitisis. In Gupte S(ed). The short textbook of pediatrics, New Delhi. Jaypee Brothers Medical Publishers 9th (millennium) Ed., pp: 204-220.
31. Hellard, M.F., M.F. Sindair, G. Hogg and C.K. Fairley, 2000. Prevalence of enteric pathogens among community based asymptomatic individuals. Journal of gastroenterology and Hepatology, 15: 290-293.
32. Meadows, M., 1990. Disorders of Gastrointestinal system. In: levene, M1 (ed). Jolly's Diseases of Children. Oxford Blackwell Scientific Publications, pp: 191-213.
33. Olstein, M.E., 2001. Surveillance of Giardia cysts in water, soil and cattle faecesInternational Journal for Parasitology, 15: 20-22.
34. Barr, S.C., 2006. Enteric protozoal infections, Giardiasis. In: Greene CE, editor. Infectious Diseases of the Dog and Cat. Philadelphia: Saunders W. B. Co., pp: 736-52.
35. Chalupka, S., 2005. Tainted water on taps, what to tell patients about preventing illness from drinking water. Trop. Med. Parasitol., 105: 50-52.