PREVALENCE AND ASSOCIATED RISK FACTORS OF GIARDIASIS IN BUFFALO CALVES OF NOMADIC COMMUNITIES, JAMMU AND KASHMIR, INDIA

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ABSTRACT

Giardiasis is a gastrointestinal disease prevalent among animals and humans worldwide. In this regard, a study was conducted to assess prevalence and associated risk factors for giardiasis in buffaloes calves reared by nomadic communities of Jammu and Kashmir, India. One hundred twenty faecal samples from buffaloes calves of age up to 1 year were collected and tested by Formol ether sedimentation and Lugol's iodine staining technique. Formol ether sedimentation gave higher prevalence 28.33% than Lugol's iodine staining technique (24.16%. The highest prevalence was observed in females (31.32%), in age group of 3 to 6 months (46.88%) and in monsoon season (53.12%). Prevalence was higher in diarrhoeal calves (32.78%) than non-diarrhoeal ones (23.72%). High risk of infection was observed in non-dewormed calves (30%), those drinking stream water (37.5%) or wallowed in contaminated water (32%) and belong to water-clogged areas (34.28%). Identification of species was done using direct immunochromatographic assay. The study highlights association of water and sanitation with *Giardia* infection in areas studied.

Keywords: *Bubalus bubalis*, buffaloes, giardiasis, prevalence, nomads

INTRODUCTION

Giardia intestinalis is a cosmopolitan intestinal parasitic disease (Thompson and Monis, 2004). The parasite has a wide host range and includes domestic (cattle, buffalo, dog, cat), wild animals and man (Kang *et al*., 1998; Barigye *et al*., 2008; Ballweber *et al*., 2010). As the pathogen is of intestinal origin, the transmission is by faeco-oral route and infection occurs on ingestion of infective cysts through contaminated food and water. The transmission of parasite from animals to humans *via* faeco-oral route is reported (Hoque *et al*., 2002; Karanis *et al*., 2006; Plutzer *et al*., 2008) and there is a tremendous potential of cross-species transmission of *Giardia* infection. The infectious dose of pathogen is very low and as less as 10 cysts can initiate infection in human and animals (Adam, 2001).

The infection and clinical disease differ with age with young children and young animals being most susceptible. In large ruminants (cattle and buffaloes), the infection of Giardia in

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diarrhoeal calves is most commonly reported at the age of 1-6 months while in non-diarrhoeal calves, it is in the age of 6 months - 1 year (Singh *et al*., 2008). Giardiasis in large ruminants with high prevalence rate may be of great concern in the areas having shallow hand pumps, pounds, canals, potentially leading to contamination of water (Das *et al*., 2015).

Buffalo rearing is an integral part of livelihood of nomads (Gujjars and Bakarwals) of Jammu and Kashmir union territory. They are scattered in the whole union territory with highest concentration in Poonch and Rajouri districts. In search of green pasture for their animals, they migrate to lower and middle mountains (like Pir Panjal) in summer (May to September) and retreat back to Jammu plains in winters (October) where they stay till April or May. These nomads live in close association with their animals and share resources for survival with animals. In this scenario, the transmission of diseases from animals to humans may happen easily. In this regard, the present study analysed buffalo calves of nomads of Jammu to determine their carriage rates for Giardia. No such study has been conducted in past on the nomads of the region.

MATERIALS AND METHODS

Faecal samples collection

A total of 120 fresh faecal samples of diarrhoeal and non diarrhoeal buffaloes calves of age up to 1 year were collected in sterile containers. For collection of samples, animal was restrained and 50 gram (if solid) or 10 ml (if liquid or semi-liquid) of faecal sample was collected directly from rectum wearing disposable gloves. After collection, the samples were brought to laboratory in ice packed containers and examined immediately under microscope or preserved in 10% formalin to be examined later on. The samples were categorized on the basis of consistency of sample, age of calf, sex of calf and season of sample collection.

Processing of faecal samples

The faecal samples were subjected to Lugol's iodine staining and Formol-ether concentration method. For Formol-ether concentration method, 5 gram of faeces was emulsified in 15 to 20 ml of normal saline followed by filtration and centrifugation 2500 rpm for 2 to 3 minutes. After decanting supernatant, 10 ml of 10% formalin was added and allowed to stand for 5 minutes. 1 to 2 ml of diethyl-ether was added to the centrifuge tube and shaken well. It was then centrifuged 2500 rpm for 10 minutes, forming four layers- a top layer of ether, a plug of debris at interface, the formalin layer and sediments at bottom. The top 3 layers were discarded, and sediment was examined on glass slide by staining with lugol's iodine solution. The samples which were positive in these tests were tested by immunochromatographic kit (Ingenasa, Spain) as per manufacturer's instructions to confirm the *Giardia* species.

Analysis of risk-factors associated with *Giardia* infection

At the time of sample collection, nomadic livestock rearers were interviewed using a semi-structured questionnaire to collect data on source of water, sanitation, management and deworming of animals.

Statistical analysis

The results were analyzed to determine the level of association between Giardia infection in

calves and various animal related and management related variables.

RESULTS AND DISCUSSIONS

Prevalence of giardiasis in buffalo calves

Out of 120 faecal samples of buffalo calves, 29 (24.16%) faecal samples were positive in Lugol's Iodine staining while 34 (28.33%) samples were positive in Formol-ether Sedimentation method. All 29 samples positive in Lugol's Iodine staining were positive in Formol-ether Sedimentation. Therefore, the results of Formol-ether Sedimentation were used further in association studies. On analysis of positive samples for *Giardia* species identification by immunochromatography kit (Ingezim, India; Ingenasa, Spain), in all samples the species found was *Giardia intestinalis*. The high prevalence in buffaloes has been reported in other studies also. Ayaz *et al.* (2010) have reported 26.11% prevalence of *Giardia* spp. in buffalo. Helmy *et al.* (2014) have reported 53% prevalence in ruminants.

Association of giardiasis with various animal-related factors

Age

In buffalo calves highest prevalence was observed in age group of 3 to 6 months (46.88%) followed by 1 to 3 months, <1 month and >6 months age groups (Table 1) although the differences were non-significant. The calves of 3 to 6 months age group were having 3 times more risk than calves of <1 month age group (Table 1) while calves of age group 1 to 3 months have almost same risk as that <1 month age group. The results are in accordance with reports of (Trout *et al.*, 2004) who mentioned highest prevalence in calves aged 3 to 7 months. Ayaz *et al.* (2010) has reported high prevalence in 0 to 6 months calves. Present work shows significant results for prevalence in 3 to 6 months aged calves as it has been reported that maternal antibodies provide protection to calves to infections for 3 months.

Sex

In study, female buffalo calves had higher prevalence (31.32%) than male calves (21.62%) although the difference was insignificant. Risk of occurrence of giardiasis in female calves was 1.65 times to male calves (Table 2). The higher prevalence in females could be due to less number of males sampled in the study compared to females as the nomadic community prefers to rear females due to their dairy occupation. Singh *et al.* (2008); Ayaz *et al.* (2010) have also reported higher prevalence in female calves. Contrarily, Suman *et al.* (2013) have observed higher prevalence in male calves.

Faecal consistency

Prevalence of giardiasis was higher in diarrhoeal calves (32.78%) as compared to non-diarrhoeal buffalo calves (23.72%) and odds ratio for giardiasis was 1.57 times in diarrheal samples to non-diarrheal samples (Table 3). Singh *et al*. (2008) have reported 34.78% prevalence in diarrhoeal group while Das *et al.* (2015) have reported 83.41% prevalence of giardiasis in diarrhoeal calves. Contrary to this, Sharma (2012) reported higher prevalence in non-diarrhoeal calves as compared to diarrhoeal calves.

Season

Seasonal analysis of data revealed that the overall prevalence of giardiasis in buffalo calves was highest during monsoon (53.12%) ($P<0.05$.

Table 1. Age wise prevalence of giardiasis in buffalo calves.

Age groups	Faecal samples			Odds ratio (95% CI)	P-value
	Examined	Positive (%)	Negative		
<1 month	27	6 (22.22)	21	1	
1-3 months	33	8 (24.24)	25	1.12 (0.33-3.74)	0.85
3-6 months	32	15 (46.88)	17	3.09 (0.99-9.68)	0.053
>6 months	28	5 (17.86)	23	0.76 (0.20-2.87)	0.68
Total	120	34	86		

Table 2. Sex wise prevalence of giardiasis in buffalo calves.

Sex	Faecal samples			Odds ratio (95% CI)	P-value
	Examined	Positive (%)	Negative		
Female	83	26 (31.32)	57	1.65 (0.66-4.10)	0.27
Male	37	8 (21.62)	29	1.00	
Total	120	34	86		

Table 3. Prevalence of giardiasis as per faecal consistency in buffalo calves.

Faecal consistency	Faecal samples			Odds ratio (95% CI)	P-Value
	Examined	Positive (%)	Negative		
Diarrhoeal	61	20 (32.78)	41	1.57 (0.70-3.50)	0.27
Non-diarrhoeal	59	14 (23.72)	45	1.00	
Total	120	34	86		

Table 4. Season wise prevalence of *Giardia* spp. in buffalo calves.

Season	Faecal samples			Odds ratio (95% CI)	P-value
	Examined	Positive (%)	Negative		
Monsoon	30	17(53.12)	13	1.00	
Post monsoon	32	4(12.5)	28	0.11 (0.03-0.39)	0.00
Winter	28	1(3.5)	16	0.05 (0.01-0.41)	0.00
Summer	30	12(40)	29	0.32 (0.12-0.82)	0.02
Total	120	34	86		

Table 5. Association between prevalence of giardiasis in buffalo calves and management factors.

Risk factors	Response	Faecal samples			Odds ratio	P-Value
		Examined	Positive (%)	Negative		
Source of drinkable water	Nallah	80	30 (37.5)	50	5.4 (1.75-16.68)	0.003
	Shallow well	40	04 (10)	36		
Bedding cleaned on regular basis	Yes	90	20 (22.22)	70	0.33 (0.14-0.78)	0.011
	No	30	14 (46.67)	16		
Deworming done in last 12 months	Yes	10	01 (10)	09	0.26 (0.03-2.13)	0.209
	No	110	33 (30)	77		
Animal wallowing in same water that man use	Yes	100	32 (32)	68	4.23 (0.92-19.36)	0.062
	No	20	02 (10)	18		
Water clogging	Yes	70	24 (34.28)	46	2.09 (0.89-4.88)	0.09
	No	50	10 (20)	40		

Chances of occurrence of disease were highest in monsoon while lowest in winter (Table 4). The present results are in conformity to that of Das *et al*. (2015) who reported highest prevalence of giardiasis in cattle calves in monsoon (30.90%). Ayaz *et al.* (2010) also reported highest prevalence (35%) during monsoon and lowest in winter (21%). It is reported that the humidity in monsoon season protects *Giardia* cysts from desiccation and increase its survivability.

Association between prevalence of giardiasis in buffalo calves and management factors

On studying association between prevalence of giardiasis in buffalo calves and management factors, it was observed that prevalence was higher when source of drinking water was Nallah (37.5%) than shallow well (10%) (P<0.05). Prevalence was higher when animals were wallowing in the water shared by the humans (32%) than not sharing (10%) (P>0.05). Prevalence was higher when clogging of water was reported (34.28%) than when not reported (20%) (P>0.05). The reason could be high chances of faecal/stool contamination in water due to open defecation practices of nomads and wallowing practices of livestock in the same water. Heitman *et al.* (2002) also observed cattle drinking water directly from rivers/streams were highly infected. Contamination of water typically increased following heavy rainfall followed by water clogging during monsoon. Runoff from unprotected areas like cow-calf pastures may also contaminate waterways with *Giardia* cysts (Heitman *et al*., 2002). Livestock wallowing/ bathing in same water in which men defecate increase the chance of occurrence of giardiasis in livestock due to direct contact with contaminated water. Further, where bedding of animals was not cleaned regularly has higher prevalence (46.67%) than were cleaned regularly (22.22%) (P<0.05).

Prevalence of giardiasis was lower (10%) when deworming was done on the farms (P>0.05) than when not done (30%). Deworming was a significant risk factor in the present study as animals dewormed showed a lower prevalence of giardiasis (10%) as compared to animals that were not dewormed (30%). O'Handley *et al.* (2001) also observed similar results by comparing two groups of Holstein calves, one given 5 mg/kg fenbendazole once daily for 3 days and other group received only sterile saline solution. The group that was regularly dewormed was *Giardia* infection free as compared to the one that was not dewormed. Fenbendazole is an effective treatment for giardiasis in calves as it eliminates trophozoites. The odds ratio calculated for various factors are shown in Table 5.

The commenced research work identified the presence of giardiasis in high proportions in the buffalo calves of nomads of Jammu region of Jammu and Kashmir and observed that the prevalence was associated with management on the farm. Thus, the people should be made aware about the importance of sanitation to control the disease.

REFERENCES

Adam, R.D. 2001. Biology of *Giardia lamblia*. *Clin. Microbiol. Rev.*, **14**(3): 447-475.

Ayaz, S., M. Ullah, F.U. Khan, A. Bibi, S. Noreen, H. Rahim and M. Akhtar. 2010. Epidemiological studies and molecular diagnosis of Giardiasis in Bovine. *Pakistan Journal of Life and Social Sciences*, **8**(2): 148-155. Available on: http://pjlss.edu.pk/pdf_files/2010_2/148-155%20(sultan-final).

pdf

Ballweber, L.R., L. Xiao, D.D. Bowman, G. Kahn and V.A. Cama. 2010. Giardiasis in dogs and cats: Update on epidemiology and public health significance. *Trends Parasitol*., **26**(4): 180-189. DOI: 10.1016/j.pt.2010.02.005

Barigye, R., N.W. Dyer, T.K. Newell, M.L. Khaitsa, J.M. Trout, M. Santin and R. Fayer. 2008. Molecular and immunohistochemical detection of assemblage E, Giardia duodenalis in scouring north Dakota calves. *Vet. Parasitol.*, **157**(3-4): 196-202. DOI: 10.1016/j.vetpar.2008.07.033

Das, M., D.K. Deka, P.C. Sarmah, S. Islam and R. Laha. 2015. Giardia duodenalis infection in dairy cattle of Assam, India. *Asian J. Anim. Vet. Adv.*, **10**(12): 911-917. DOI: 10.3923/ajava.2015.911.917

Heitman, T.L., L.M. Frederick, J.R. Viste, N.J. Guselle, U.M. Morgan, R.C.A. Thompson and M.E. Olson. 2002. Prevalence of Giardia and Cryptosporidium and characterization of *Cryptosporidium* spp. isolated from wildlife, human, and agricultural sources in the North Saskatchewan River Basin in Alberta, Canada. *Can. J. Microbiol*., **48**(6): 530-541. DOI: 10.1139/w02-047

Helmy, Y.A., C. Klotz, H. Wilking, J. Krücken, K. Nöckler, G. Von Samson-Himmelstjerna, K.H. Zessin and T. Aebischer. 2014. Epidemiology of Giardia duodenalis infection in ruminant livestock and children in the Ismailia province of Egypt: Insights by genetic characterization. *Parasite Vector.*, **7**(231): 1-11. Available on: https://parasitesandvectors.biomedcentral.com/track/pdf/10.1186/1756-3305-7-321.pdf

Hoque, M.E., V.T. Hope, T. Kjellström, R. Scragg and R. Lay-Yee. 2002. Risk of giardiasis in Aucklanders: A case-control study. *Int. J. Infect. Dis.*, **6**(3): 191-197. DOI: 10.1016/s1201-9712(02)90110-4

Kang, G., M.S. Mathew, D.P. Rajan, J.D. Daniel, M.M. Mathan, V.I. Mathan and J.P. Muliyil. 1998. Prevalence of intestinal parasites in rural Southern Indians. *Trop. Med. Int. Healht*, **3**(1): 70-75. DOI: 10.1046/j.1365-3156.1998.00175.x

Karanis, P., I. Sotiriadou, V. Kartashev, C. Kourenti, N. Tsvetkova and K. Stojanova. 2006. Occurrence of Giardia and Cryptosporidium in water supplies of Russia and Bulgaria. *Environ. Res*., **102**(3): 260-271. DOI: 10.1016/j.envres.2006.05.005

O'Handley, R.M., A.G. Buret, T.A. McAllister, M. Jelinski and M.E. Olson. 2001. Giardiasis in dairy calves: Effects of fenbendazole treatment on intestinal structure and function. *Int. J. Parasitol*., **31**(1): 73-79. DOI: 10.1016/s0020-7519(00)00148-x

Plutzer, J., P. Karanis, K. Domokos, A. Törökné and K. Márialigeti. 2008. Detection and characterisation of Giardia and Cryptosporidium in Hungarian raw, surface and sewage water samples by IFT, PCR and sequence analysis of the SSUrRNA and GDH genes. *Int. J. Hyg. Envir. Heal*., **211**(5-6): 524-533. DOI: 10.1016/j.ijheh.2008.04.004

Sharma, S. 2012. *Epidemiological investigation of Giardiosis in Animals and Humans*, M.V. Sc. Thesis, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, India.

Singh, B.B., R. Sharma, J.K. Sharma, H.S. Banga, H. Kumar, R.S. Aulakh and J.P.S. Gill. 2008. Prevalence of Giardia intestinalis infection in cattle. *J. Vet. Parasitol*., **22**: 91-92.

Suman, M., M. Alam, S. Pun, A. Khair, S. Ahmed and R. Uchida. 2013. Prevalence of *Giardia lamblia* infection in children and calves in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, **9**(2): 177-182.

Thompson, R.C. A. and P.T. Monis. 2004. Variation in giardia: Implications for taxonomy and epidemiology. *Adv. Parasitol.*, **58**: 69-137. DOI: 10.1016/S0065-308X(04)58002-8

Trout, J.M., M. Santín, E. Greiner and R. Fayer. 2004. Prevalence of Giardia duodenalis genotypes in pre-weaned dairy calves. *Vet. Parasitol.*, **124**(3-4): 179-186. DOI: 10.1016/j.vetpar.2004.07.010