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Concurrent Infection of Gastro-Intestinal Parasites and Bacteria Associated with Diarrhea in Bengal Goats in Bangladesh

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ABSTRACT: Hundred diarrhoeic kids' faecal samples were examined of which 64% and 98% kids are affected with parasites and bacteria respectively. Around 59.7% and 61.22% had single, 31.34% and 37.76% had dual and only 4.48% and 1.02% had triple concurrent infection respectively in parasite and bacteria associated diarrhoeic kids. Analysis of the results of bacterio-parasitic enteropathogens revealed that highest percentage (64%) kids were infected with concurrent parasitic and bacterial infection but the faecal samples of four diarrhoeic kids showed negative for both bacteria and parasitic infections. Highest of gastro-intestinal nematodes (GIN) infection (48%) was recorded in kids followed by *Eimeria* sp. (27%), *Toxocara vitulorum* (14%) and lowest (1%) with each of the *Strongyloides* sp., *Paramphistomum* sp. and *Moniezia* sp. The clinical *Toxocara vitulorum* infection first recorded at the first of 15 days, GIN at 30 days, *Strongyloides* sp. at 45 days, *Paramphistomum* sp. at 150 days and *Eimeria* sp. infection at 25 days. Similarly, the rate of *Bacillus* infection (87%), was found highest followed by *Escherichia coli* (37%), *Staphylococcus* (9%), and lowest of *Salmonella* (5%) infection. Although *Bacillus* and *E. coli* recorded first time at 10 days and *Staphylococcus* at 30 days of age but *Salmonella* was recorded at the age of 60. A characteristic age specific prevalence of both parasitic and bacterial enteropathogens was observed and it was found that the severity of infection was increasing with age for both parasitic and bacterial infection and the ratio of all the infections were almost similar.

KEYWORDS: Parasite, bacteria, diarrhea, Bacterial infection, Bengal goat.

1 INTRODUCTION

Diarrhoea, caused by different enteropathogens has been recognized as a major clinical problem for calves in Bangladesh [7]. Debnath *et al*., [6] reported 52% loss of kid production both through morbidity, and mortality caused by gastroenteritis in Bangladesh. Enteropathogens include bacteria, viruses, fungi, protozoa, and helminthes have been recognized to be associated with diarrhoea [14]. Reports on enteropathogens associated with kid diarrhoea are very limited in Bangladesh. Therefore, an attempt was made to determine the parasites, and bacteria associated with kid diarrhea in Bangladesh. At present in our sub-continent, importance on livestock health, and production is not significant. As a result, protein deficiency becomes an unsolved problem. Therefore, enhancement on small ruminants production especially goat in small scale agriculture assume the great significance [9]. So current study will designed with the following objectives:

1. To find out the definite gastro-intestinal parasites, and bacteria responsible for goat/kid diarrhoea so that appropriate regulatory actions are taken.
2. To enhance the dynamism of goat farming that will alleviate the poverty as well as boost up the national economy.

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2 MATERIALS & METHODS

This study was carried out randomly selected 100 clinically diarrhoeic kids aged between 10 to 180 days of Bengal goats in Bangladesh Livestock Research Institute Goat Farm during two years periods from May 2004 to April 2006 at Goat & Sheep Production Research Division (GSPRD), Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341, Bangladesh.

2.1 SAMPLE COLLECTION

Faecal samples were selected as experimental samples. 20 gram (gm) faecal samples of each of the selected diarrhoeic kids were collected directly from the rectum aseptically in sterile vials and transferred to the laboratory for Bacterio-parasitological examinations and occasionally stored at – 20^0C until tested. Special care was always taken to avoid contamination as possible.

2.2 MEDIA PREPARATION

Different commercially available media were prepared according to the direction of the manufacturer's for culture, and sub-culture of the organisms in order to get pure culture by proper isolation, and identification.

2.3 PARASITOLOGICAL EXAMINATION

Most of the tapeworm, and ascarid infection in kids was recognized with naked eye at the time of collection of faecal samples by the presence of segments, and adult ascarid worms respectively. The tapeworm segments were grinded with mortar and pestle with small amount of water and then the fluid was examined under microscope to confirm the *Moniezia* sp. Each of the collected faecal samples was examined on conventional direct smear method and followed by sedimentation methods to detect parasitic eggs which were identified by their morphological features as described by Samad [15]. As it was difficult to differentiate the eggs of different species of nematode parasites and accordingly they were grouped as Gastro-intestinal Nematode (GIN) parasites. Faecal samples that were found positive for parasitic infection on direct conventional method were also examined by quantitative Stoll's dilution technique for counting egg per gram (epg) of faeces for helminthes' eggs and Mac-Master method for counting *Eimeria* oocysts as described by Samad [15] & epg ≥ 200 and oocysts ≥ 5000/g of faeces were considered significant for clinical infections [17].

2.4 BACTERIOLOGICAL EXAMINATION

All the 100 randomly selected diarrhoeic faecal samples of kids were examined for isolation and identification of bacteria. Each of the faecal samples was streaked on Nutrient agar, and Blood agar to promote bacterial growth. The colonies on primarily cultures were repeatedly sub-cultured by Streak Plate Method [4] until pure culture with homogenous colonies were obtained. Media like Nutrient agar, Blood agar, *Staphylococcus* Medium No-110, Eosin Methylene Blue (EMB), MacConkey agar, Triple Sugar Iron (TSI) media, *Salmonella-Shigella* agar (SS) were used for sub-cultures. Bacteria were identified by Gram Staining [5], cultural, morphological characters, and biochemical (Sugar fermentation with five basic sugar e.g. Dextrose, Sucrose, Lactose, Maltose, and Mannitol), Catalase, Coagulase & IMViC utilization) tests as described by Buxton and Fraser [3].

2.5 STATISTICAL ANALYSIS

The results were analyzed statistically by using chi-square test for significances [8].

3 RESULTS & DISCUSSION

Bacterio-parasitological methods were used to determine the gastro-intestinal bacteria and parasites associated with diarrhoea in kids. The age of kids and pathogen factors (single and concurrent infection) were assessed for the occurrence of the disease. The faecal examination of 100 diarrhoeic kids revealed that 64% kids were affected with different types of GI parasites (Table.1) and 98% kids had different types of bacterial infections (Table.2). These observations support the earlier reports of Hossain *et al*., [10] who reported 58.2% mortality of kids due to GI helminthiasis and Samad [16] reported 82.62% clinically sick kids had GI parasitic infection. Of the 64% kids affected with GI parasites, of which 59.7% had single, 31.34% had dual, and only 4.48% three types of concurrent parasitic infection (Table.1). This finding supports the earlier report of Samad [16] who reported 63.32% single, 33.74% dual, and only 2.94% triple GI parasitic infection in calves. Similarly, of the

98 diarrhoeic kids affected with bacterial enteropathogens, of which 61.22 % had single type, 37.76% had two types, and only 1.02% had three types of infection (Table.2).

Table 1. Pattern of occurrence of single and mixed gastro-intestinal parasites in diarrhoeic kids

S/N	Parasites	Age in days			Total (N=100)	
		10-30 (N=16)	31-90 (N=37)	91-180 (N=47)	No	%
1	*Toxocara vitulorum* (TV)	-	-	-	-	**59.7***
2	GI Nematodes (GIN)	02	04	26	32	
3	*Strongyloides* (S)	-	01	-	01	
4	*Eimeria* sp. (E)	02	03	02	07	
Total (Single infection)		**04**	**08**	**28**	**40**	
5	GIN + E	04	04	02	10	**31.34**
6	TV + E	01	06	-	07	
7	GIN + *Moniezia* sp. (M)	-	-	01	01	
8	GIN + *Paramphistomum* sp.	-	-	02	02	
9	GIN + TV	-	01	-	01	
Total (Concurrent - 2 types)		**05**	**11**	**05**	**21**	
10	TV + GIN + E	-	02	-	02	**04.48**
11	GIN + *Trichuris* sp. + E	-	-	01	01	
Total (Concurrent - 3 types)		**-**	**02**	**01**	**03**	
Total(Concurrent infection)		**05**	**13**	**06**	**24**	**35.82**
Overall (single + mixed)		**09 (13.43%)**	**21 (31.34%)**	**34 (50.75%)**	**64**	**64.00**

N= No. of kids examined ***** *Significant at (p <0.01)*

Analysis of the result showed that kids aged between 10 to 30 days had low level of both parasitic (13.43%) and bacterial (16.33%) infections in comparison to 31 to 90 days (31.34% and 39.80% respectively), and 91 to 180 days (50.75% and 43.88% respectively) (Table.1 and Table.2). these findings are in conformity with the earlier report of Samad *et al.* [19] who reported low level of parasitic infection in calves up to 30 days of age in comparison to higher age group.

Table 2. Pattern of occurrence of bacterial enteropathogens in diarrhoeic kids

S/N	Bacteria	Age in days			Total (N=100)	
		10-30 (N=16)	31-90 (N=37)	91-180 (N=47)	No	%
1	*Escherichia coli* (EC)	01	04	03	08	**61.22**
2	*Bacillus* sp. (B)	09	18	24	51	
3	Staphylococcus sp. (Staph)	-	01	-	01	
Total (Single infection)		**10**	**23**	**27**	**60**	
4	B + EC	05	10	10	25	**37.76**
5	B + Staph.	-	03	01	04	
6	B + *Salmonella* sp.	-	01	04	05	
7	EC + Staph.	01	01	01	03	
Total (Concurrent - 2 types)		**06**	**15**	**16**	**37**	
8	EC + B + Staph.	-	01	-	01	**01.02**
Total (Concurrent – 3 types)		**-**	**01**	**-**	**01**	
Total (Concurrent infection)		**06**	**16**	**16**	**38**	**38.78**
Total positive		**16 (16.33)**	**39 (39.80)**	**43 (43.88)**	**98**	**98.00**
Total negative		**01(50.00)**	**-**	**01 (50.00)**	**02**	**02.00**
Over all (single + mixed)		**17 (17.34%)**	**39 (39.80%)**	**44 (44.90%)**	**100**	**100 (100)**

N = No. of kids examined

It indicates that the rates of infection of enteropathogens are increased with the increase of age of kids which is probably proportionate to the period of exposure to infection, and incubation period of the disease. The results of concurrent infection with GI parasites and bacteria showed that only 18.18% kids had single bacterial infection, 42.42% had two types, 30.30% three types, 08.08% four types and only 01.01% five types of infection of infections of either with bacteria or parasites or both (Table.3). It also appears that single bacterial enteropathogen could be associated with diarrhoea in kids (18.18%) but significantly higher rate of diarrhoeic kids (81.81%) were associated with concurrent infection, either with multiple bacteria or parasites or both (Table.3). From the results, it may be concluded that GI parasite may not be only responsible alone for kids' diarrhoea.

Table 3. Pattern of occurrence of parasitic and bacterial enteropathogens in diarrhoeic kids

S/N	Bacteria	Age in days			Total (N=100)*	
		10-30 (N=16)	31-90 (N=37)	91-180 (N=47)	No	%
1	*Bacillus* sp. (B)	02	06	07	15	
2	*Escherichia coli* (EC)	01	-	02	03	**18.18**
Total (Single infection)		**03**	**06**	**09**	**18**	
3	B + EC	04	05	01	10	
4	B + *Staphylococcus* sp. (Staph.)	-	01	-	01	
5	B + GI Nematodes (GIN)	02	04	13	19	
6	EC + Staph.	-	01	01	02	
7	EC + *Toxocara vitulorum* (TV)	-	01	-	01	**42.42**
8	EC + GIN	-	01	01	02	
9	GIN + *Paramphistomum* sp.	-	-	01	01	
10	B + *Eimeria* sp. (E)	02	02	02	06	
Total (Concurrent - 2 types)		**08**	**15**	**19**	**42**	
11	B + EC + Staph.	-	01	-	01	
12	B + S + GIN	-	-	03	03	
13	B + Staph + GIN	-	01	-	01	
14	B + EC + GIN	-	01	06	07	
15	B + EC + TV	-	02	01	03	
16	B + TV + E	01	01	-	02	
17	B + *Moniezia* sp. + GIN	-	-	01	01	**30.30**
18	B + E + GIN	03	03	01	07	
19	Staph. + EC + GIN	-	01	01	02	
20	Staph. + GIN + E	-	01	-	01	
21	EC + TV + E	-	01	-	01	
22	Staph. + EC + E	-	01	-	01	
Total (Concurrent – 3 types)		**04**	**13**	**13**	**30**	
23	B + EC + GIN + E	-	01	01	02	
24	B + Staph. + GIN + E	-	01	-	01	
25	B + EC + TV + E	01	02	-	03	**08.08**
26	B + S + TV + E	-	01	-	01	
27	B + E + GIN + TV	-	01	-	01	
Total (Concurrent – 4 types)		**01**	**06**	**01**	**08**	
28	B + EC + GIN + E + *Trichuris* sp.	-	-	01	01	**01.01**
Total (Concurrent – 5 types)		**-**	**-**	**01**	**01**	
Total (Concurrent infection)		**13**	**33**	**35**	**81**	**81.81**
Total positive (Single + mixed)		**16 (16.33)**	**39 (39.79)**	**43 (43.88)**	**99**	**99.00**

****** One kid (14 day-old) was negative for both parasite & bacteria, 18 kids had single bacteria, 14 had concurrent bacteria, one had concurrent parasites and 66 had concurrent bacteria and parasitic infections.*

3.1 GASTRO-INTESTINAL PARASITIC INFECTION

Microscopic examination of faecal samples showed higher rate of infection with GI nematodes (48.0%), followed by *Eimeria* sp. (27.0%), *Toxocara vitulorum* (14.0%) and lowest of 1% with each of the *Strongyloides* sp., *Paramphistomum* sp. and *Moniezia* sp. (Table.4).

The species of the GI nematode parasites associated with diarrhoea in kids have not been identified in this study and presence of any nematode eggs other than *Toxocara, Strongyloides* and *Trichuris* infection were considered to be GI nematodes. The 48% clinical occurrence of GI nematodes recorded in calves up to 180 days of age supports the earlier report of Samad [16] who reported 32.73% prevalence of parasitic gastro-enteritis caused by nematode parasites in calves. It appears that the clinical GI nematode infection started at the age of 30 days (10.42%) and then a steady increased with the age and highest rate was found at 91 to 180 days (64.58%) of age (Table.4).

In this study, clinical *Eimeria* sp. were recorded 27% in kids aged between 25 to 180 days but higher infection rate (59.26%) was observed in kids aged between 31 to 90 days (Table.4). These findings support the report of Radostits *et al.* [14] who reported 15 to 20 days prepatent and 6 to 30 days incubation period of *Eimeria* sp. infection.

Table 4. Age wise occurrence of parasitic and bacterial enteropathogens associated with diarrhoea in kids

S/N	Enteropathogens	Age in days			Total (N=100)	
		10-30 (N=16)	31-90 (N=37)	91-180 (N=47)	No	%
A. Parasites						
1	*Toxocara vitulorum*	01[a] (07.14)	12(85.71)	01 (07.14)	14	**14.00**
2	GI Nematodes	05[b] (10.42)	12 (25.00)	31 (64.58)	48	**48.00**
3	*Strongyloides* sp.	-	01[c] (100)	-	01	**01.00**
4	*Paramphistomum* sp.	-	-	01[d] (100)	01	**1.00**
5	*Moniezia* sp.	-	-	01[e] (100)	01	**1.00**
6	*Eimeria* sp.	06[f] (22.22)	16 (59.26)	05 (18.52)	27	**27.00**
B. Bacteria						
1	*Staphylococcus* sp.	01[x] (11.11)	06 (66.67)	02 (22.22)	09	**09.00**
2	*Bacillus* sp.	13[y] (14.94)	32 (36.78)	42 (48.28)	87	**87.00**
3	*Escherichia coli*	07[y] (18.92)	14 (37.84)	16 (43.24)	37	**37.00**
4	*Salmonella* sp.	-	01[z] (20.00)	04 (80.00)	05	**05.00**

N= No. of kids examined, GI= Gastro-intestinal, - = Negative, 1st recorded at the age of: [a]15 days, [b]30days, [c]45days, [d]150days, [e]91days, [f]25 days, [x]30 days, [y]10 days, [z]60 days.

Although *T. vitulorum* infection was recorded at the 15th days of age but higher infection rate was recorded in kids aged between 31 to 90 days (85.71%) of age in comparison to 10 to 30 (7.14%) and 90 to 180 (7.14%) days (Table.4). This finding supports the earlier report of Karim *et al.*, [11] who reported 44% subclinical *T. vitulorum* infection with infection rate in calves aged between 1 to 3 months (60%) than 4 to 6 months (28%) old calves. The findings of occurrence of clinical *T. vitulorum* infection in neonatal calves within the 1st two weeks of life suggest that *T. vitulorum* larvae have been passed to newborn calves through colostrum/milk [12]. This result also supports the description of Radostits *et al.* [14] who reported that *T. vitulorum* larvae are passed in great numbers in the colostrum 2 to 5 days after calving, worms age matured in the intestine of the calves by 10 days of age and eggs are passed by 3 weeks and then the adult worms are expelled from the intestine by 5 month of age, and for this reason, Toxocariasis has been considered as calf hood disease.

Only one case of *Strongyloides* infection in calf was recorded at the age of 45 days (Table.4). This finding supports the earlier observations of Moyo *et al.* [13] and Bharkad *et al.,*[2] who reported *Strongyloides* infection in calves of 2 to 4 months and up to 3 months of age, respectively.

Paramphistomum infection was recorded in only one calf at the age of 150 days (Table.4). The presence of adult *Paramphistomum* in the rumen has said not to be elicited any clinical response but in massive infestations are associated with the clinical signs [14]. However, kid affected with adult *Paramphistomum* (epg 700) which resulted fetid diarrhoea and ill-health.

Moniezia infection was recorded in one calf at the age of 91 days (Table-4). This finding supports the Radostits *et el.* [14] who described the signs of Moniezia infestation are restricted chiefly to animals less than six month of age. The clinical manifestation of *Moniezia* infestation have been described to be associated with diarrhoea and ill- health which are in conformity with the earlier report of Samad *et al.* [18] who reported and outbreak of Monieziasis with diarrhoea and ill-health in calves.

3.2 BACTERIAL ENTEROPATHOGENS

Bacteriological examination of faecal samples showed that 98% kids had either single or concurrent infection with four different types of bacteria (Table.2). Higher infection rate was recorded with *Bacillus* sp. (87%), followed by *E. coli* (37%), *Staphylococcus* sp. (9.0%) and lowest with *Salmonella* sp. (5%) (Table.4). The *Bacillus* sp. and *E. coli* organisms were recorded in the faeces from the 10 days age of kids, and *Staphylococcus* sp. from 30 days of age but *Salmonella* sp. from 60 days of age in diarrhoeic kids (Table.4). These rates of infection support the earlier reports of Debnath *et al.* [7] who reported 20% *E. coli* and 3% *Salmonella* sp. infection in diarrhoeic calves. The results also supports the findings of *Haque et al.,* [9] who reported *Salmonella* sp. (5.0%), *Staphylococcus* sp. (10.0%), *Escherichia coli* (25.0%) and *Bacillus* sp. (85.0%) in diarrhoeic goats. Amin *et al.*, [1] also reported the prevalence of enteropathogenic *E. coli* in 10%, 8.57% and 9.38% diarrhoeic calves less than one year of age in three different dairy farms in Bangladesh.

4 CONCLUSION

Bengal goats are potential and economic livestock of Bangladesh. A large number of goats are decreasing in regular basis due to diarrhoea. This study was carried out to identify the main etiological agents of diarrhoea and it was found that *Toxocara vitulorum*, GIN, *Strongyloides* S., *Eimeria* sp., *Moniezia* sp., *Paramphistomum* sp., *Trichuris* sp., *Escherichia coli*, *Bacillus* sp., *Staphylococcus* sp., *Salmonella* sp. altogether play a vital role for goat/kid diarrhoea. Results of this study will help to develop an effective treatment method of goat/kid diarrhoea against those organisms.

REFERENCES

[1] Amin, M.M., Rahman, M.H., Ali, M.R., Huq, M.I. and Chowdhury, K.A., "Diarrhoea due to *E. coli* in calves," *Bangladesh Vet. J.,* 22: 7-12, 1988.

[2] Bharkad, G.P., Deshpande, P.D. and Narladkar, B.W., "Gastro-intestinal parasites in bovine calves in Marathwada," *J. Vet. Parasitol.*, 13:143-146, 1999.

[3] Buxton, A. and Fraser, S., "*Animal Microbiology*," Blackwell Scientific Publications, vol. I, Oxford, London, Edinburgh, Melbourne, pp. 205-228, 1977-1987.

[4] Cheesbrough, M., "Culturing of Anaerobes," In : *Medical Laboratory Manual for Tropical Countries*, 2nd edn., Butterworth Co., Kent, UK., 1985.

[5] Cowan, S.T., *Cowan and Steel's Manual for Identification of Medical Bacteria,* 2nd edn., Cambridge University Press, London, 1985.

[6] Debnath, N.C, Sill, B.K., Selim, S.A., Pradhan, M.A.M. and Howliader, M.M.R., "A retrospective study of calf mortality and morbidity on smallholder traditional farms in Bangladesh," *Prev. Vet. Med.,* 9:1-7, 1990.

[7] Debnath, N.C., Huq, M.I. and Rahman, A. A, "microbial investigation of neonatal calf diarrhoea in Bangladesh," *Indian J. Anim Sci*., 57: 1035-1038, 1987.

[8] Gupta, S.P., *Statistical Methods*, 16th edn. Sultan Chand & Son, New Delhi, 1982.

[9] Haque, M.M., Ershaduzzaman, M., Islam, M.E., Das, T. and Rahman, M.M., "Isolation and identification of etiological agents from diarrhoeic goats," *Asian Journal of Animal and Veterinary Advances,* 2(1):1-8, 2007.

[10] Hossain, M.I., Hossain, M.M., Hannen, B.M.A. and Wadud, A.C., "A note on mortality in young calves due to gastro-intestinal helminthiasis in and around BAU Campus," *Bang. J. Anim. Sci.,* 21:127-129, 1992.

[11] Karim, R., Rafiq, K., Mostofa, M. and Hasan, Q., "Therapeutic trials of certain anthelminthics against Ascariasis in calves," *Bangladesh Vet. J.,* 32:71-73, 1998.

[12] Mia, S.A., Dewan, M.L., Uddin, M., and Chowdhury, M.U.A., "The route of infection of buffalo calves by *Toxocara (Neoascaris) vitulorum*," *Trop. Anim. Hlth Prod,* 7: 153-156, 1975.

[13] Moyo, D.Z., Bwangamoi, O., Hendrikx, W.M.L. and Eysker, M., "The epidemiology of gastro-intestinal nematode infections in communal cattle and commercial beef cattle on the Highveld of Zimbabwe," *Vet. Parasitol*., 67: 105-120, 1996.

[14] Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.M., *Veterinary Medicine,* 9th edn., W.B. Saunders, London, 2000.

[15] Samad, M.A., *Poshu Palon O Chikitsavidya,* 2nd edn., LEP Pub. No. 08, BAU Campus, Mymensingh, Bangladesh, 2001.

[16] Samad, M.A., "Epidemiological studies on gastro-intestinal parasites in calves under traditional management in Bangladesh," *Bangladesh Vet. J.,* 35: 9-18, 2001.
[17] Samad, M.A., *Veterinary practitioner's Guide*, 1st pub., LEP Pub., No. 07, BAU Campus, Mymensingh, Bangladesh, 2000.
[18] Samad, M.A., Haque, A.K.M.F., Rahman, A., Ali, K.M. and Sen, M.M., "Clinico-pathological report of an acute outbreak of Monieziasis in calves," *Bangladesh Vet. J.,* 11:81-84, 1997.
[19] Samad, M.A., Hossain, K.M.M., Saha, S. and Islam, M.A., "Concurrent infection of gastro-intestinal parasites and bacteria associated with diarrhoea in calves," *Bangladesh Vet. J.,* 99:30-35, 2003.