

CONCURRENT INFECTION OF GASTRO-INTESTINAL PARASITES AND BACTERIA ASSOCIATED WITH DIARRHOEA IN CALVES

M. A. Samad, K. M. M. Hossain¹, M. A. Islam and S. Saha¹

Department of Medicine, Department of Microbiology and Hygiene¹, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh – 2202, Bangladesh

ABSTRACT

Bacterio-parasitological examination of faecal samples of randomly selected 100 diarrhoeic calves aged between 10 to 180 days, were carried out to determine the parasites and bacteria associated with diarrhoea in calves. These calves (either sexes and both local & cross-bred) were brought for treatment at the Bangladesh Agricultural University Veterinary Clinic, Mymensingh during the two years period from June 1999 to May 2001. Of the 100 faecal samples examined, of which 67% calves affected with different parasites and 98% with different bacteria. Of the 67 and 98 calves affected with parasites and bacteria, of which 65.67% and 61.22% had single, 29.85% and 37.76% had dual and only 3.33% and 1.02% had triple concurrent infection, respectively. Analysis of the results of bacterio-parasitic enteropathogens of 100 calves revealed that highest percentage (66%) calves were infected with concurrent parasitic and bacterial infection, followed by 18% with single bacteria, 14% with concurrent bacteria, and only 1% with concurrent parasitic infection but the faecal sample of one diarrhoeic calf showed negative for both bacteria and parasitic infections. Highest rate of gastro-intestinal nematodes (GIN) infection (48%) was recorded in calves, followed by *Eimeria* spp. (27%), *Toxocara vitulorum* (14%) and lowest (1%) with each of the *Strongyloides*, *Paramphistomum* and *Moniezia* sp. The clinical *T. vitulorum* infection was first recorded at the age of 15 days, GIN at 30 days, *Strongyloides* at 45 days, *Paramphistomum* at 150 days and *Eimeria* infection at 25 days. Similarly, the rate of *Bacillus* infection (87%) was found highest, followed by *E. coli* (37%), *Staphylococcus* (9%) and lowest of *Salmonella* (5%) infection. Although the *Bacillus* and *E. coli* recorded first time at the 10 days and *Staphylococcus* at 30 days age of calves but *Salmonella* was recorded at the age of 60 days. A characteristic age specific prevalence of both parasite and bacterial enteropathogens was observed. It may be concluded from this study that concurrent infections with parasites or bacteria or both are mainly associated with diarrhoea in calves.

Key words : Concurrent infection, GI parasites, bacteria, diarrhoea, calves

INTRODUCTION

Diarrhoea, caused by different enteropathogens has been recognized as a major clinical problem for calves in Bangladesh (Debnath *et al.*, 1987). Debnath *et al.* (1990) reported 52% calf morbidity and mortality caused by gastro-enteritis in Bangladesh. Enteropathogens include bacteria, viruses, fungi, protozoa and helminths have been recognized to be associated with diarrhoea (Radostits *et al.*, 2000). Reports on enteropathogens associated with calf diarrhoea are very limited from Bangladesh (Samad *et al.*, 1977 ; Debnath *et al.*, 1987 ; Amin *et al.*, 1988). Therefore, an attempt was made to determine the parasites and bacteria associated with calf diarrhoea in Bangladesh.

MATERIALS AND METHODS

This study was carried out on randomly selected 100 clinically diarrhoeic calves, aged between 10 to 180 days, of either sexes and both the local and cross-bred, which were brought for treatment at the BAU Veterinary Clinic, Mymensingh during two years period from June 1999 to May 2001. Faecal samples of each of the selected 100 diarrhoeic calves were collected directly from the rectum aseptically in sterile vials and transferred to the laboratory for examination and occasionally stored at - 20 °C until tested.

Parasitological examination

Most of the tapeworm and ascarid infection in calves was recognized with naked eye at the time of collection of faecal samples by the presence of segments and adult ascarid worms in the faeces, 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 sample was examined on conventional direct smear method and followed by sedimentation methods to detect the parasitic eggs which were identified by their morphological features as described by Samad (2001a). As it was difficult to differentiate the eggs of different species of nematode parasites and accordingly, they were grouped as GI nematode parasites. Faecal samples 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 helminths eggs and MacMaster method for counting *Eimeria* oocyst as described by Samad (2001a), and epg \geq 200 and oocyst \geq 5000 / g of faeces were considered significant for clinical infections (Samad, 2000).

Bacteriological examination

All the 100 randomly selected diarrhoeic faecal samples of calves were examined for isolation and identification of bacteria. Each of the faecal samples was streaked on Nutrient agar and Blood agar to promote growth of bacteria. The colonies on primarily cultures were repeatedly subcultured by streak plate method (Cheesbrough, 1985) 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, Salmonella-Shigella agar (SSA) were used for subcultures. Bacteria were identified on cultural, morphological characters and biochemical (sugar fermentation, catalase, coagulase & IMViC utilization) tests as described by Cowan (1985).

Statistical analysis

The results were analyzed statistically by using Chi-square test for significance (Gupta, 1982).

RESULTS AND DISCUSSION

Bacterio-parasitological methods were used to determine the gastro-intestinal bacteria and parasites associated with diarrhoea in calves. The age of calves and pathogen factors (single and concurrent infection) were assessed for the occurrence of the disease. The faecal examination of 100 diarrhoeic calves revealed that 67% calves were affected with different types of GI parasites (Table 1) and 98% calves had different types of bacterial infections (Table 2). These observations support the earlier reports of Hossain *et al.* (1992) who reported 58.2% mortality of calves due to GI helminthiasis and Samad (2001b) reported 82.62% clinically sick calves had GI parasitic infection. Of the 67% calves affected with GI parasites, of which 65.67% had single, 29.85% dual and only 3.33% three types of concurrent parasitic infection (Table 1). This finding supports the earlier report of Samad (2001b) who reported 63.32% single, 33.74% dual and only 2.94% triple GI parasitic infection in calves. Similarly, of the 98 diarrhoeic calves affected with bacterial enteropathogens, of which 61.22% had single type, 37.76% had 2 types and only 1.02% had three types of infection (Table 2). Analysis of the result showed that calves aged between 10 to 30 days had low level of both parasite (11.94%) and bacterial (16.33%) infections in comparison to 31 to 90 days (37.31% & 39.80%) and 91 to 180 days (50.75% & 43.88%) respectively (Table 1 & 2). These findings are in conformity with the earlier report of Samad (2001b) who reported low level of parasitic infection in calves up to 30 days of age in comparison to higher age group. It indicates that the rate of infection of enteropathogens increased with the increase of age of calves which is probably proportionate to the period of exposure to infection and incubation period of the disease.

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

S/N	Parasites	Age in days			Total (n = 100)	
		10 - 30 (n = 16)	31 - 90 (n = 37)	91 - 180 (n = 47)	No.	%
1.	<i>Toxocara vitulorum</i> (TV)	-	03	01	04	
2.	GI nematodes (GIN)	02	04	26	32	
3.	<i>Strongyloides</i> sp. (S)	-	01	-	01	
4.	<i>Eimeria</i> sp. (E)	02	03	02	07	
	Total (Single infection)	04	11	29	44	65.67*
5.	GIN + E	03	05	02	10	
6.	TV + E	01	06	-	07	
7.	GIN + <i>Moniezia</i> sp. (M)	-	-	01	01	
8.	GIN + <i>Paramphistomum</i> sp.	-	-	01	01	
9.	GIN + TV	-	01	-	01	
	Total (Concurrent - 2 types)	04	12	04	20	29.85
10.	TV + GIN + E	-	02	-	02	
11.	GIN + <i>Trichuris</i> sp. + E	-	-	01	01	
	Total (Concurrent - 3 types)	-	02	01	03	03.33
	Total (Concurrent infection)	04	14	05	23	34.33
Overall (single + mixed)		08 (11.94%)	25 (37.31%)	34 (50.75%)	67	67.00

n = No. of calves examined.

*Significant at (p < 0.01)

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

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

n = No. of calves examined.

Analysis of the results of concurrent infection with GI parasites and bacteria showed that only 18.18% calves had single bacterial infection, 42.42% had two types, 30.30% three types, 8.08% four types and only 1.01% five types of infections of either with bacteria or parasites or both (Table 3). It appears from the summary of the Table 3 that 18.14% diarrhoeic calves were affected with single types of bacteria, 14% with mixed bacteria, only 1% with concurrent two types of parasites but 66% calves had concurrent infection with multiple bacteria and parasites (Table 3). Although single bacterial enteropathogen could be associated with diarrhoea in calves (18.18%) but significantly higher rate of diarrhoeic calves (81.82%) were associated with concurrent infection either with multiple bacteria or parasites or both (Table 3). It may be concluded from the results of this study that GI parasites may not be responsible alone for the production of clinical diarrhoea in calves.

Gastro-intestinal parasitic infections

Microscopic examination of faecal samples of 100 diarrhoeic calves aged between 10 to 180 days showed higher rate of infection with GN 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 calves 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 GIN recorded in calves up to 180 days of age supports the earlier report of Samad (2001b) 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 increase with the age and highest rate was found at 91 to 180 days (64.58%) of age (Table 4).

Reports on the incidence of clinical bovine coccidiosis are very scanty in Bangladesh. However, Mondal and Qadir (1978) and Karim *et al.* (1990) reported subclinical infection of coccidiosis with identification of species of causative *Eimeria*. Six species of *Eimeria* identified in cattle of Bangladesh were : *E. zurnii*, *E. bovis*, *E. auburnensis*, *E. cylindrica*, *E. subspherica* and *E. bukidnonensis*. In this study, clinical coccidiosis was recorded in 27% calves aged between 25 to 180 days but higher infection rate (59.26%) was observed in calves aged between 31 to 90 days (Table 4). These findings support the report of Radostits *et al.* (2000) who reported 15 to 20 days prepatent and 6 to 30 days incubation period of *Eimeria* infection.

Although the *T. vitulorum* infection was recorded at the 15th days of age but higher infection rate was recorded in calves 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.* (1998) who reported 44% subclinical *T. vitulorum*

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

S/N	Parasites	Age (days)			Total (n = 100)*	
		10 - 30 (n = 16)	31 - 90 (n = 37)	91 - 180 (n = 47)	No.	%
1.	<i>Bacillus</i> sp. (B)	02	06	07	15	
2.	<i>Escherichia coli</i> (EC)	01	-	02	03	
	Total (Single infection)	03	06	09	18	18.18
3.	B + EC	04	05	01	10	
4.	B + <i>Staphylococcus</i> sp. (Staph.)	-	01	-	01	
5.	B + <i>Eimeria</i> sp. (E)	02	02	02	06	
6.	B + GI nematodes (GIN)	02	04	13	19	
7.	EC + Staph.	-	01	01	02	
8.	EC + <i>Toxocara vitulorum</i> (TV)	-	01	-	01	
9.	EC + GIN	-	01	01	02	
10.	GIN + <i>Paramphistomun</i> sp.	-	-	01	01	
	Total (Concurrent - 2 types)	08	15	19	42	42.42
11.	B + S + GIN	-	-	03	03	
12.	B + Staph. + EC	-	01	-	01	
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 + <i>Moniezia</i> sp. + GIN	-	-	01	01	
18.	B + E + GIN	03	03	01	07	
19.	Staph. + EC + GIN	-	01	01	02	
20.	Staph. + EC + E	-	01	-	01	
21.	Staph. + GIN + E	-	01	-	01	
22.	EC + TV + E	-	01	-	01	
	Total (Concurrent - 3 types)	04	12	14	30	30.30
23.	B + EC + GIN + E	-	01	01	02	
24.	B + Staph. + GIN + E	-	01	-	01	
25.	B + EC + TV + E	01	02	-	03	
26.	B + S + TV + E	-	01	-	01	
27.	B + E + GIN + TV	-	01	-	01	
	Total (Concurrent - 4 types)	01	06	01	08	08.08
28.	B + EC + GIN + <i>Trichuris</i> sp + E	-	-	01	01	
	Total (Concurrent - 5 types)	-	-	01	01	01.01
	Total (Concurrent infection)	13	33	35	81	81.82
Total +ve (single + mixed)		16 (16.33)	39 (39.79)	44 (43.88)	99	99.00

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

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 the *T. vitulorum* larvae have been passed to newborn calves through colostrum / milk (Mia *et al.*, 1975). This result also supports the description of Radostits *et al.* (2000) who reported that *T. vitulorum* larvae are passed in great numbers in the colostrum 2 to 5 days after calving, worms are 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, toxocarasis has been considered as calfhood disease.

Only one case of *Strongyloides papillosus* infection in calf was recorded at the age of 45 days (Table 4). This finding supports the earlier observations of Moyo *et al.* (1996) and Bharkad *et al.* (1999) who reported *S. papillosus* infection in calves of 2 to 4 months and up to 3 months of age, respectively.

Parasitic and bacterial enteropathogens

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

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.	<i>Toxocara vitulorum</i>	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.	<i>Strongyloides</i> sp.	-	01 ^c (100)	-	01	01.00
4.	<i>Paramphistomum</i> sp.	-	-	01 ^d (100)	01	01.00
5.	<i>Moniezia</i> sp.	-	-	01 ^e (100)	01	01.00
6.	<i>Eimeria</i> sp.	06 ^f (22.22)	16 (59.26)	05 (18.52)	27	27.00
B. Bacteria						
1.	<i>Staphylococcus</i> sp.	01 ^x (11.11)	06 (66.67)	02 (22.22)	09	09.00
2.	<i>Bacillus</i> sp.	13 ^y (14.94)	32 (36.78)	42 (48.28)	87	87.00
3.	<i>Escherichia coli</i>	07 ^z (18.92)	14 (37.84)	16 (43.24)	37	37.00
4.	<i>Salmonella</i> sp.	-	01 ^z (20.00)	04 (80.00)	05	05.00

n = No. of calves examined. GI = Gastro-intestinal. - = Negative

1st recorded at the age of : ^a15 days, ^b30 days, ^c45 days, ^d150 days, ^e91 days, ^f25 days, ^x30 days, ^y10 days, ^z60 days

Paramphistomum infection was recorded in only one calf at the age of 150 days (Table 4). The presence of adult paramphistomes in the rumen has said not to be elicited any clinical response but in massive infestations are associated with the clinical signs (Radostits *et al.*, 2000). However, this calf 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 al.* (2000) 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.* (1977) who reported an outbreak of monieziasis with diarrhoea and ill-health in calves.

Bacterial enteropathogens

Bacteriological examination of 100 diarrhoeic faecal samples of calves showed that 98% calves 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%) of organisms (Table 4). The *Bacillus* sp. and *E. coli* organisms were recorded in the faeces from the 10 days age of calves, and *Staphylococcus* sp. from 30 days of age but *Salmonella* sp. from 60 days of age in diarrhoeic calves (Table 4). These rates of infection support the earlier reports of Debnath *et al.* (1987) who reported 20% *E. coli* and 3% *Salmonella* infection in diarrhoeic calves. Amin *et al.* (1988) also reported the prevalence of enteropathogenic *E. coli* in 10%, 8.57% and 9.38% diarrhoeic calves under one year of age in three different dairy farms in Bangladesh.

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