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PREVALENCE AND FACTORS AFFECTING THE PARASITIC INFECTIONS IN CALVES AT SELECTED AREAS OF BANGLADESH

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Abstract

Gastrointestinal parasitic infections are considered as one of the major impediments in profitable livestock farming in subtropical and tropical countries. The present study was carried out to investigate the prevalence of gastrointestinal (GI) parasites and to determine the effects of different factors in the occurrences of GI parasitic infections in calves. A cross-sectional study, including 413 fecal samples from calves, was conducted in Pabna, Sirajgonj and Gazipur districts of Bangladesh. The samples were examined using standard coprological techniques like sedimentation and floatation techniques, and lugol's iodine and modified Ziehl-Neelson staining. The overall prevalence of gastrointestinal parasitism was 45.3% and commonly identified parasites were *Toxocara* spp. (20.3%), *Strongyloides* spp. (3.9%), *Fasciola* sp. (1.0%), *Moniezia* spp. (1.5%), *Giardia* sp. (10.4%) and *Cryptosporidium* spp. (7.0%). Among the factors, the age, sex and health status had significant effects on the GI parasitic infections in calves. Therefore, special care such as routine fecal examination for parasitism and proper deworming program should be taken to maintain good health and husbandry of calves for profitable livestock production.

Keywords: Gastrointestinal parasites, prevalence, calves, coprological techniques.

Introduction

Gastrointestinal (GI) parasitism is a disease caused by different genera of parasites that inhabit the digestive tract of animals, causing inappetence, anemia, diarrhea, poor growth, and economic losses in the herds. Basically, GI parasitism in livestock is caused by helminths and protozoa (Pinilla León *et al.,* 2019). These infections are rarely associated with high mortality and estimated that about 10% animals die annually due to parasitic diseases in the world (Chavhan *et al*., 2008). However, their effects are usually characterized by reduced livestock productivity as indicated by a slower growth rate, low milk production, low body condition score (BCS) as well as additional therapeutic cost (Charlier *et al.,* 2015). The productivity losses through reduced feed intake and decreased efficiency in feed utilization due to subclinical or chronic infections are also hindering profitable livestock industry (Akanda *et al.,* 2014). In addition, these infections enhance susceptibility to secondary infections and

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losses resulting from condemnation of carcasses and organs (Hendawy, 2018; Gunathilaka *et al.,* 2018).

One of the major constraints in livestock production is parasitic infection (Jabber and Green, 1983). The prevalence of parasitic infection depends on ecology, geographical and climatic condition prevailing in Bangladesh (Hossain *et al.,* 2004). The farmers usually rear their cattle under traditional husbandry practices. Nutritional status of the animals in general is not satisfactory as they are over-worked but under-fed or half-fed, which makes the animal susceptible to diseases including different parasitic diseases. About 50% calves until 1-year of age die due to GI parasitism (Sardar *et al.,* 2006). Rahman and Ahmed (1991) reported that calves gained body weight by 400 gm/day when treated for parasitic diseases compared to 200 gm/day in non-treated calves. It was also reported that anthelmintic treated calves reached to sexual maturity in 24 months compared to 36-40 months by non-treated calves. Afazuddin (1985) estimated an annual loss of 0.1 million Bangladesh Taka due to parasitic diseases in Military Farm, Savar, Dhaka. Unfortunately, in Bangladesh the parasitic diseases are neglected or overlooked sometimes since the infected animals show little or no clinical signs (Alim *et al.,* 2012).

In this study, we selected three districts namely, Pabna, Sirajgonj and Gazipur. The geo-climatic conditions of these three districts and the water logging and low lying areas of Pabna and Sirajgonj districts are expected to favors the growth, development and survival of various parasites or their hosts. Besides, there are several factors, such as breed, age, sex, nutritional and immune status which may influence the occurrences of GI parasitic infections. Although, previous studies in some selected areas of Bangladesh revealed wide prevalence of GI parasitism in livestock (Paul *et al.,* 2016; Ahmed *et al.,* 2015; Islam *et al.,* 2014), no precise report on the infections is available in calves of these areas. Considering this, the present research work was undertaken to determine the prevalence of GI parasitic infections of calves in Pabna, Sirajgonj and Gazipur districts and to evaluate the effect of geographic location, breed, age, sex, nutritional status and fecal consistency on occurrence of GI parasitism.

Materials and Methods

Study area

The study was conducted in different locations of Sirajgonj, Pabna and Gazipur districts. The milk pocket areas of Sirajgonj and Pabna were chosen, because the farmers over there are mostly dependent on dairy farming and parasitic infection is more common in these areas. On the other hand, the industrial zone Gazipur was selected to compare the prevalence and diversity of GI parasitic infections with that found in another two districts. Among the 24 Upazilas of these three districts, six Upazilas were selected for this study, such as Sadar and Santhia Upazilas of Pabna district; Sadar, Ullapara and Shahjadpur Upazilas of Sirajgonj district; and Sadar Upazila of Gazipur district (Fig. 1).

Study design and data collection

A cross-sectional study was conducted in randomly selected dairy farms of study areas during the period from January 2018 to June 2018. The minimum sample size

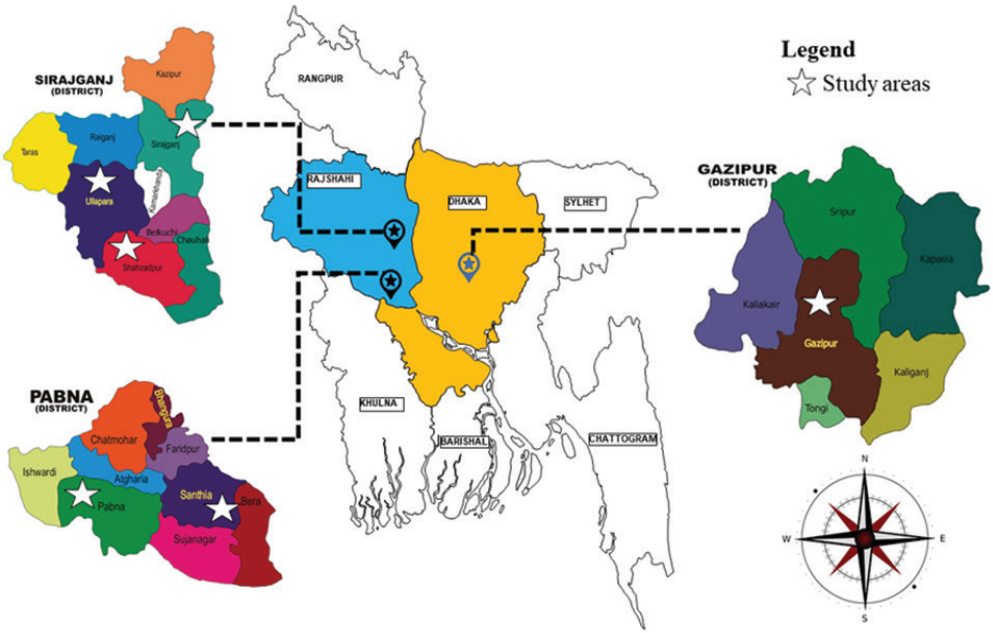

Fig. 1. Sampling locations of Pabna, Sirajgonj and Gazipur districts. The three districts have 24 Upazilas, of which this study included Sadar and Santhia Upazilas of Pabna; Sadar, Ullapara and Shahjadpur Upazilas of Sirajgonj; and Sadar Upazila of Gazipur.

(n=368.64) was calculated using the formula, $n= Z^2P(1-P)/d^2$ based on a prevalence of 60% with desired precision of 5% at 95% confidence level (Thrusfield, 2007). A total of 413 calves were selected randomly for the collection of fecal samples ageing up to 8 months. During fecal sample collection, a pretested questionnaire was used to record the age, sex, health condition, breed, and level of consistency of fecal materials of calves. The calves were divided into three age groups viz. ≤ 3 months, >3 to ≤ 6 months and > 6 to ≤ 8 months; different sex viz. male and female; different health status group viz. poor and normal; different breeds viz. Non-descriptive indigenous and crossbred. The ages of the calves were determined by interviewing the farmers or by examining the teeth. The health status of the calves was determined by visual observation. Well fleshed calves having no bony prominence and glistening hair coat was considered as calves with normal health. Calves with externally visible ribs and other bony prominence and rough hair coat were considered as poor health conditioned (Pinilla *et al*., 2018).

Fecal sample collection and preservation

Single fresh fecal sample from each consenting calf was collected in labeled and sterile stool containers containing SAAF solution (Sodium Acetic Acid Formalin). The fecal samples was taken either directly from the rectum of the animals or from the ground immediately after defecation using disposable gloves. Before collection, the animals were restrained properly and all possible hygienic measures including wearing apron, hand gloves and gumboot were taken to avoid contamination.

About 20-25 gram of feces was collected from each calf and transported to the laboratory in ice box, and examined as early as possible.

Examination of fecal samples

The fecal samples were analyzed using standard parasitological screening techniques namely, sedimentation followed by floatation technique to detect the eggs, cysts and oocysts of parasites (Taylor *et al.,* 2016). For identification of *Giardia* cysts and *Cryptosporidium* oocysts, lugol's iodine and modified Ziehl-Neelson staining were performed, respectively. The eggs, cysts and oocysts of parasites were identified from their morphological characters, using a light optical microscope with a magnification of 10 x and 40 x.

Simple Sedimentation Technique

About 10 grams of faces and 100 ml of saline solution were taken in a glass cylinder. The mixture was thoroughly stirred to make a uniform suspension of the fecal particles. The suspension was then allowed to pass through a sieve (30-50 meshes to the inch) into another glass cylinder and then allowed to stand for half an hour. The supernatant fluid was carefully poured off and a small amount of sediment was taken out with the help of a medicinal dropper and was placed on a glass slide. A coverslip was placed on it and care was taken to avoid bubble formation between the glass slide and the coverslip. The slide was then placed under a compound microscope and examined with low power objective 10x.

Flotation technique

Flotation procedure was performed using Sheather's Sucrose solution. The fecal pellet was resuspended in 10 ml of Sheather's solution (specific gravity 1.27 g/ml) and mixed thoroughly. The mixture was increased up to the brim of the centrifuge tube and centrifuged for 10 minutes at 4000 rpm. The downward force created by the centrifugal spinning enhanced the buoyancy of the eggs in the viscous solution and drove them to the surface meniscus where they were concentrated and resulted in greater parasite recovery. Examination of a few drops of the fluid from the topmost layer revealed the eggs and oocysts/cysts (Dryden *et al.,* 2005).

Lugol's iodine staining

Direct smear from the sediment of each concentrated fecal sample was prepared on a clean glass slide, diluted with a drop of Lugol's iodine, covered with coverslip and finally examined under light microscope at 40X magnification to observe the *Giardia* cysts (Hendrix, 2002).

Modified Ziehl-Neelson staining

Thin smears were prepared from sediments of concentrated fecal samples and air-dried. The smears were fixed with absolute methanol for 5 minutes, air dried and stained with carbol-fuchsin (0.34% fuchsin and 4% w/v phenol) for 30 minutes. Smears were washed with tap water and decolorized with 1% acid-alcohol (1 ml hydrocloric acid and 99 ml of 96% ethanol) for 2 minutes; washed with tap water and counterstained with 1% methylene blue for another 2 minutes, rinsed again in tap water and air-dried. The stained smears were examined by microscope using oil immersion objective to screen oocysts of *Cryptosporidium* (Tahvildar-Biderouni and Salehi, 2014).

Statistical analysis

The data generated from the questionnaire and parasite identification were recorded in the Statistical Package for the Social Sciences (SPSS 20.0). Descriptive statistics and the Chi-square test were done to determine the significant effects of different explanatory variables on percentage values of parasitism.

Results and Discussion

Overall prevalence of GI parasitic infections in calves

Out of the 413 calves examined through fecal examination, 187 were found infected with one or more species of GI parasites indicating an overall prevalence of 45.3% (Table 1). The identified helminths were the snail-borne trematode, namely, *Fasciola* sp. (1.0%), two species of protozoa, namely, *Giardia* sp. (10.4%) and *Cryptosporidium* spp. (7.0%), two species of nematodes, namely, *Toxocara* spp. (20.3%) and *Strongyloides* spp. (3.9%) and one cestode *Moniezia* spp. (1.5%) (Fig. 2). However, mixed infection was found only in case of nematodes (*Toxocara* spp. and *Strongyloides* spp.) with the prevalence of 1.2%.

From this study, it was observed that the prevalence of T*oxocara* spp. (20.3%) was the highest whereas, *Fasciola* sp. (1.0%) infection was the lowest among the parasitic infections in calves (Table 1). The overall prevalence of GI parasitism was lower than the findings of Paul *et al.* (2016) and Aktaruzzaman *et al.* (2013) who reported that 72.65% and 76.9% cattle were infected with various helminths at Sylhet and Sirajgonj, respectively in Bangladesh. Similarly, Bhattacharyya and Ahmed (2005) and Singh *et al.* (2008) recorded 65.2% and 80.0% incidence of gastrointestinal helminthes, respectively in cattle in India. The variation between the present and earlier results might be due to the differences among the geographical locations and climatic conditions of the study areas, feeding, management and genetic variation in host resistance as well as a gradual increase in awareness of farmers about routine deworming in study areas. Similar to this study, calves were mostly infected with *Toxocara* spp. and

Table 1. Overall prevalence of GI parasitic infection in calves

Types of Parasites	Name of Parasites	No. Infected (N=413)	Prevalence (%)
Nematode	*Toxocara* spp.	84	20.3
	Strongyloides spp.	16	3.9
	Mixed *(Toxocara* spp. and *Strongyloides* spp.)	5	1.2
Trematode	*Fasciola* sp.	4	1.0
Cestode	*Moniezia* spp.	6	1.5
Protozoa	*Giardia* sp.	43	10.4
	Cryptosporidium spp.	29	7
Overall		187	45.3

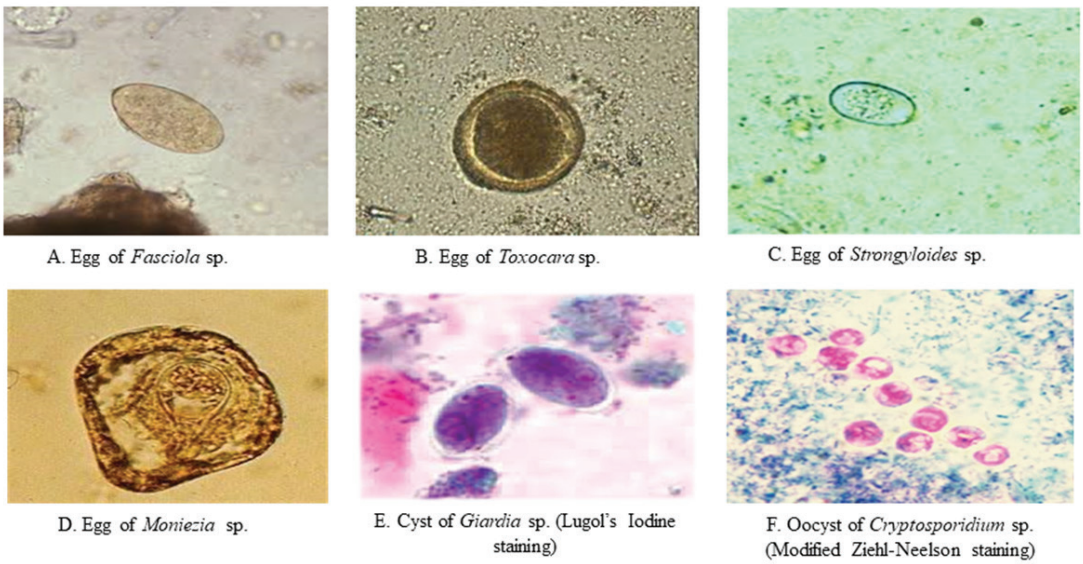

Fig. 2. Identified GI parasites in this study.

high prevalence of infection might be related to prenatal infection with 3rd larval stage, and poor hygienic condition during post-natal period (Miller *et al*., 2013). However, similar types of parasites as reported in this study were detected by different scientists in different areas with variable rate of infections (Ahmed *et al.,* 2015; Islam *et al.,* 2014; Fayer, 2010; Xiao, 2010). In Bangladesh, most of the calves in rural areas are reared in scavenging or semi scavenging system, where they graze on the fields. This type of practice may favor the parasitic infestations in calves.

Diversity of GI parasitic infection according to study location

The prevalence of different GI parasitic infections in three study districts varied from 44.5 to 46.3%. The lowest parasitic infection (44.5%) was recorded in Pabna district though infection by different species of parasites in study areas was statistically not significant (Table 2). The slight difference in the prevalence of GI parasitic infections might be due to variation in geo-climatic conditions of these areas of study.

Prevalence of GI parasitic infections in relation to age

The prevalence of GI parasitic infections varied significantly (p= 0.037) among different age groups of calves and the highest infection was recorded in calves of >3 to ≤ 6 months (54.4%) and lowest in calves of ≤ 3 months (36.6%) of age (Table 3).

The prevalence of *Toxocara* spp. infection was the highest in calves of >3 to ≤6 months (29.4%) and the lowest in the calves of >6 to ≤8 months (8.2%). The protozoan infection by *Giardia* sp. (16.4%) and *Cryptosporidium* spp. (8.2%) were the highest in the calves of >6 months to ≤8 months. Prevalence of snail-borne trematode infection was found

Table 2. Diversity of GI parasitism in calves in different study areas

Type of Parasites	Study area			*p*-value
	Sirajgonj (%) (n= 163)	Pabna (%) (n= 209)	Gazipur (%) (n= 41)	
Toxocara spp.	31 (19.0)	42 (20.1)	11 (26.8)	0.54
Strongyloides spp.	7 (4.3)	9 (4.3)	0 (0.0)	0.40
Mixed *(Toxocara* spp. and *Strongyloides* spp.)	4 (2.5)	0 (0.0)	1 (2.4)	0.08
Fasciola sp.	1 (0.6)	2 (1.0)	1 (2.4)	0.57
Moniezia spp.	3 (1.8)	3 (1.4)	0 (0.0)	0.68
Giardia sp.	18 (11.0)	20 (9.6)	5 (12.2)	0.83
Cryptosporidium spp.	11 (6.7)	17 (8.1)	1 (2.4)	0.42
Total	75 (46.0)	93 (44.5)	19 (46.3)	

Table 3. Prevalence of GI parasitic infections in calves in relation to Age

Type of Parasites	Age category			*p*-value
	≤ 3 months (%) (n=172)	>3 to ≤ 6 months (%) (n= 180)	> 6 to ≤ 8 months (%) (n= 61)	
Toxocara spp.	26 (15.1)	53 (29.4)	5 (8.2)	
Strongyloides spp.	7 (4.1)	7 (3.9)	2 (3.3)	
Mixed *(Toxocara* spp. and *Strongyloides* spp.)	2 (1.2)	3 (1.7)	0 (0.0)	
Fasciola sp.	0 (0.0)	1 (0.6)	3 (4.9)	0.037
Moniezia spp.	0 (0.0)	5 (2.8)	1 (1.6)	
Giardia sp.	16 (9.3)	17 (9.4)	10 (16.4)	
Cryptosporidium spp.	12 (7.0)	12 (6.7)	5 (8.2)	
Total	63 (36.6)	98 (54.4)	26 (42.6)	

to increase with the increase of age and was highest at age of >6 to ≤8 months (4.9%). *Fasciola* sp. was not recorded in calves less than 3 months of age. Susceptibility of calves to *Moniezia* spp. was highest in calves of >3 to ≤6 months (2.8%) and absent in calves under 3 months (Table 3). This result is inclined with the results by Raza *et al.* (2010) and Samad *et al.* (2004) who reported that these parasites are mostly prevalent in young age. The cause of this high prevalence in young cattle might be due to sudden exposure to grassland containing huge number of eggs of parasites, and possibly due to lack of necessary protective immunity of the calves. *Fasciola* sp. and *Moniezia* spp. were not observed in calves under 3 months of age. This might be the consequences of feeding habit of calves and time requirement for completing the life cycle of these parasites. However, Rahman

and Mondal (1983) found heavy infection of *Fasciola* sp. in cattle of 2-3 years of age than in the young cattle.

Sex-wise distribution of GI parasites in calves

In the present study, prevalence of GI parasitic infection was observed significantly (p= 0.029) higher in male (48.4%) than in female calves (42.5%) (Table 4). In males, the highest prevalent parasite was *Toxocara* spp. (18.8%) followed by *Giardia* sp. (12.0%), *Cryptosporidium* spp. (9.9%), *Strongyloides* spp. (4.7%), *Fasciola* sp. (1.6%), and *Moniezia* spp. (1.0%).

In female calves, the highest prevalence was recorded for *Toxocara* spp. (21.7%) and lowest for *Fasciola* sp. (0.5%) (Table 4). Similar to this finding, higher prevalence was reported in male animals than in females by some other studies in Bangladesh, Pakistan and Ethiopia (Paul *et al.*, 2016; Hailu *et al.,* 2011; Ibrahim *et al.,* 2008).On the other hand, higher rate of parasite infection in female animals than in male was also reported (Das *et. al.,* 2010; Islam and Taimur, 2008). However, Siddiki *et al*. (2010) observed that both male and female Red Chittagong Cattle breed and crossbred animals were equally susceptible to parasitic infections. The higher percentage of infection in the male cannot be explained exactly, but it might be due to the neglected attitude of the farmers toward the management of male animals since many of the farms target milk production thereby focusing more on the health of females. In addition, higher feed and water intake might make the male individual more susceptible to any infection (Paul *et al.,* 2016).

Variation in GI parasitism according to breed

In this study, prevalence of parasitic infection was more common in cross breed calves (48.0%) than in indigenous calves (34.5%). But the difference was not significant (p= 0.816) (Table 5). In both breeds, the highest prevalence of GI parasitic infection was recorded for *Toxocara* spp. (21.6, 15.5%)

Table 4. Prevalence of GI parasitic infections in calves of different sexes

Type of Parasites	Sex category		*p*-value
	Male (%) (n= 192)	Female (%) N= 221	
Toxocara spp.	36 (18.8)	48 (21.7)	0.029
Strongyloides spp.	9 (4.7)	7 (3.2)	
Mixed *(Toxocara* spp. and *Strongyloides* spp.)	1 (0.5)	4 (1.8)	
Fasciola sp.	3 (1.6)	1 (0.5)	
Moniezia spp.	2 (1.0)	4 (1.8)	
Giardia sp.	23 (12.0)	20 (9.0)	
Cryptosporidium spp.	19 (9.9)	10 (4.5)	
Total	93 (48.4)	94 (42.5)	

Table 5. Prevalence of GI parasitism in calves according to breed

Type of Parasites	Breed		*p*-value
	Indigenous (%) (n= 84)	Crossbred (%) (n= 329)	
Toxocara spp.	13 (15.5)	71 (21.6)	0.816
Strongyloides spp.	3 (3.6)	13 (4.0)	
Mixed *(Toxocara* spp. and *Strongyloides* spp.)	0 (0.0)	5 (1.5)	
Fasciola sp.	1 (1.2)	3 (0.9)	
Moniezia spp.	1 (1.2)	5 (1.5)	
Giardia sp.	5 (6.0)	38 (11.6)	
Cryptosporidium spp.	6 (7.1)	23 (7.0)	
Total	29 (34.5)	158 (48.0)	

and the lowest for *Fasciola* sp. (0.9%, 1.2%). Similar findings had been described by Gadre (2007) who reported that the infection rates with GI parasitic infections in cross-bred cattle were relatively higher than in local dairy animals. This study revealed that the infection rate with *Toxocara* spp. was comparatively higher in cross-breed (21.6%) than in the indigenous calves (15.5%) which are almost similar to the earlier report by Roy (2010).

Holstein Frisian and Jersey are usually adapted in countries having relatively low temperature with minimal chances of parasitic exposure. The parasitic ecology and reproduction are closely related to an optimal environmental condition, which is not normally common in these countries. But, Bangladesh is a tropical country with hot-humid environment which is favorable for parasite reproduction. For this reason, crossbred animals in Bangladesh become readily infected by parasites and different predisposing factors including managing of these animals in parasitic load environment further worsen the condition.

Effect of health status on GI parasitic infections

Health status of calves had significant (p= 0.004) effect on the occurrence of GI parasitism and infections were higher in poor health conditioned calves (76.5%) than that of normal conditioned (39.1%). In calves with poor health, the highest prevalence was recorded in case of *Toxocara* spp. (44.1%) followed by *Giardia* sp. (11.8%), *Strongyloides* spp. (7.4%), *Fasciola* sp. (4.4%), *Cryptosporidium* spp. (4.4%), and *Moniezia* spp. (1.5%). In healthy calves, the highest prevalence was found for *Toxocara* spp. (15.7%) and the lowest for *Fasciola* sp. (0.3%) (Table 6).

This finding coincides with the result reported by Ilyas *et al.* (2016) and Alim *et al.* (2012). Malnourished animals are more susceptible to any infection as they are immune compromised. It appears that malnutrition in animals increases their susceptibility to the parasitic infection (Biswas *et al.,* 2014). It may also happen due to the fact that the poor

Table 6. Health status related prevalence of GI parasitism in calves

Type of Parasites	Health status		*p*-value
	Normal (%) (n= 345)	Poor (%) (n= 68)	
Toxocara spp.	54 (15.7)	30 (44.1)	0.004
Strongyloides spp.	11 (3.2)	5 (7.4)	
Mixed *(Toxocara* spp. and *Strongyloides* spp.)	3 (0.9)	2 (2.9)	
Fasciola sp.	1 (0.3)	3 (4.4)	
Moniezia spp.	5 (1.4)	1 (1.5)	
Giardia sp.	35 (10.1)	8 (11.8)	
Cryptosporidium spp.	26 (7.5)	3 (4.4)	
Total	135 (39.1)	52 (76.5)	

and weak animals, as a result of any other causes, are not able to resist the challenge of parasitic infection and become easily infected.

Relationship between fecal consistency and GI parasitic infections in calves

Calves with loose feces had more parasitic infection (56.5%) than the calves with formed (21.7%) and soft (18.7%) feces, however the difference was not significant (Table 7). Among the parasites, *Toxocara* spp. was more common in calves with each type of feces (loose = 21.7%, formed=18.7% and soft=21.0%) whereas *Fasciola* sp. and *Moniezia* spp. were found in calves with soft (0.6% and 2.9%, respectively) and loose feces (4.8% and 1.6%, respectively). Loose feces is a common clinical finding in many parasitic as well as bacterial and viral diseases that make animal immune-compromised and vulnerable. For this reason, calves with loose feces might have more parasitic infection than others.

Table 7. Prevalence of GI parasitic infections in calves based on fecal consistency

Type of Parasites	Fecal Consistency			*p*-value
	Formed (%) (n=180)	Soft (%) (n= 171)	Loose (%) (n= 62)	
Toxocara spp.	39 (21.7)	32 (18.7)	13 (21.0)	0.424
Strongyloides spp.	7 (3.9)	7 (4.1)	2 (3.2)	
Mixed *(Toxocara* spp. and *Strongyloides* spp.)	2 (1.1)	2 (1.2)	1 (1.6)	
Fasciola sp.	0 (0.0)	1 (0.6)	3 (4.8)	
Moniezia spp.	0 (0.0)	5 (2.9)	1 (1.6)	
Giardia sp.	16 (8.9)	17 (9.9)	10 (16.1)	
Cryptosporidium spp.	12 (6.7)	12 (7.0)	5 (8.1)	
Total	76 (42.2)	76 (44.4)	35 (56.5)	

Conclusions

GI parasitic infections are common among calves in Pabna, Sirajgonj and Gazipur districts. The highest prevalence was found for *Toxocara* spp. and the lowest for *Fasciola* sp. It was also found that age, sex and health status had significant effects on the prevalence of GI parasitism in calves. Parasitic diseases pose great effects on health and production of animals. Economic losses due to mortality and morbidity in calves per year in Bangladesh may be determined by further studies. More extensive research including molecular epidemiology may be conducted to develop cost effective sustainable control strategies against GI parasitism.

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