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PREVALENCE OF *GIARDIA LAMBLIA* INFECTION IN CHILDREN AND CALVES IN BANGLADESH

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ABSTRACT

Giardia lamblia is highly infectious protozoan parasite capable of causing gastrointestinal illness in both humans and animals. The objective of this study was to determine the prevalence of *Giardia lamblia* infection in children < 5 years old and calves. Enzyme Linked Immunosorbent Assay (ELISA) has been used for the detection of *Giardia lamblia*. A total of 266 children and 15 calves diarrheic fecal samples were tested for *Giardia lamblia* during January 2011 to May 2012. The prevalence of *Giardia lamblia* infection among children was 3.8% while 13.3% in calves. *Giardia lamblia* was highest in children between 24 and 60 months of age (8.7%). *Giardia lamblia* infection was higher in male (4.7%) than in female (2.0%). Male calves (14.3%) have slightly higher prevalence than female calves (12.5%). The highest prevalence (33.3%) of *Giardia lamblia* infection in calves was between the ages 6 and 9 months. This is the first study to determine the prevalence of *Giardia lamblia* infection in calves using ELISA method in Bangladesh. A larger scale study is needed for accurate estimates of prevalence of *Giardia lamblia* to undertake an appropriate control strategy in future.

Key words: *Giardia lamblia*; Children diarrhea; Calves diarrhea; ELISA; Prevalence.

INTRODUCTION

The intestinal protozoan parasite, *Giardia labmlia* (Syn. *Giardia intestinalis*, *Giardia duodenalis*) has recently emerged as an important cause of diarrhoea in humans and animals. It is distributed worldwide and presents high levels of genetic diversity. In developing countries, about 200 million people have symptomatic giardiasis with some 500,000 new cases reported each year (WHO, 1996). The disease is most common in areas where sanitation and hygiene are poor (Nygard *et al.,* 2006). Increased prevalence in human as well as in some of the surrounding animals offers an emerging concern about the role played by some animals in human giardiasis (Olson, 2004). Each individual eliminates up to 900 million cysts per day. Higher prevalence is found in tropical and subtropical areas, in urban than in rural where *Giardia labmlia* affects up to 30% of the population (Minvielle *et al.,* 2004). A previous study has found *Giardia lamblia* in 68% of children aged between 2 to 8 months in Bangladesh (Hall, 1994). Haque *et al.* (2003) reported 11.08% *Giardia lamblia* infection in 2–5 years old group in Mirpur, an urban slum area in Dhaka. In Bangladesh several studies has been performed to determine the prevalence of *Giardia lamblia* (Haque *et al.,* 2003; Alam *et al.,* 2011) by direct microscopic examination. Very few researches have been conducted by modern diagnostic techniques like immunofluorescence assay, enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR). There is a need for a sensitive and specific diagnostic procedure for screening *Giardia lamblia*. ELISA has greater sensitivity and specificity than the conventional diagnosis techniques (eg. direct microscopic examination) (McGlade *et al.,* 2003). ELISA test detect soluble antigens in the feces. A number of commercial coproantigen capture enzyme linked immunosorbent assay (ELISA) based tests (Dryden *et al.,* 2006) have been shown to have higher sensitivities than zinc flotation and microscopy for the detection of *Giardia lamblia*. There is no published data on the prevalence of *Giardia lamblia* infection using ELISA in calves in Bangladesh. The present research work was undertaken to determine the prevalence of *Giardia lamblia* infection in children and calves in Bangladesh using Sandwich-ELISA.

MATERIALS AND METHODS

Sample collection

The present study was carried out during January 2011 to May 2012 in the Laboratory, Department of Medicine, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Stool specimens were collected from hospitalized children admitted to Institute of Children and Maternity Hospital (ICMH), Matuail, Dhaka. Calves specimens were collected from Bangladesh Agricultural University (BAU) and surrounding areas of Mymensingh. Specimens were collected from rectum of calves inserting fingers equipped with thin rubber hand gloves and were collected in sterile screw capped containers. Five to ten grams of stool specimen was collected. Precautions were taken to avoid contamination from one specimen to the other. The samples were then transferred to the container containing ice bag and stored at -200C as quickly as possible and transported to the laboratory of the department of Medicine at BAU. Information about the age, sex, clinical signs and symptoms and date of collection was recorded.

Preparation of Sample (Fresh/Frozen Stools)

Frozen specimens were thawed at room temperature and 1:4 dilutions of the specimens were prepared in eppendorf tube by using 0.3 ml of dilution buffer (approximately 0.1 g). In case of watery specimens, 0.1 ml of specimen was added to 0.3 ml dilution buffer in eppendorf tubes. In case of semisolid specimens, samples were centrifuged at 2000-3000 rpm for 5-10 minutes to produce clear supernatant.

Method

Enzyme Linked Immunosorbent Assay (Sandwich, Antibody Coated Plate, Diagnostic Automation, Inc. USA)

Interpretation of results

Results were estimated visually by naked eye.
Reactive: Sample well that is obviously more yellow than the negative control well.
Non-reactive: Sample well that is not obviously more yellow than the negative control well.

Statistical analysis

The data was entered in Microsoft Excel 2007 and transferred to R 2.14.2 (The R Foundation for Statistical Computing, Vienna, Austria) for descriptive and analytical statistics. Bivariable logistic regression method was used to determine any association between age and sex of humans with *Giardia lamblia* infection in R.

RESULTS AND DISCUSSION

A total of 266 children and 15 calves specimens were examined to determine the prevalence of *Giardia lamblia* infection in Bangladesh. The prevalence of *Giardia lamblia* infection among hospitalized diarrheic children was found to be 3.8% and 13.3% in calves. The prevalence of *Giardia lamblia* infection in children and calves is presented in Table 1.

Table 1. Prevalence of *Giardia lamblia* infection in human and calves

Species	No. tested	No. positive	**Prevalence** (%)	95% Confidence Interval
Human	266	10	3.8	1.8 - 6.8
Calves	15	2	13.3	1.7 - 40.5

The prevalence of *Giardia lamblia* infection was highest (8.7%) in children between 24 to 60 months of age although it was not statistically significant. The age wise distribution of *Giardia lamblia* infection in children is presented in Table 2.

Table 2. Age specific distribution of *Giardia lamblia* infection in hospitalized children with diarrhea in ICMH, Dhaka

Age in groups (months)	No. tested	No. positive	**Prevalence** (%)	95% Confidence Interval (CI) of **Prevalence**	Odds Ratio (OR)	95% CI of OR
< 6 months	11	0	0	0 – 28.5	Ref.	Ref.
6-12 months	141	3	2.1	0.4 – 6.1	2.34	0, Infinity
13 -24 months	91	5	5.5	1.8 – 12.4	6.08	0, Infinity
25-60 months	23	2	8.7	1.1 - 28.0	1.01	0, Infinity
Total	**266**	**10**	**3.8**	**1.8 - 6.8**	-	-

Table 3. Age specific distribution of *Giardia lamblia* infection in calves

Age in groups (months)	No. tested	No. positive	**Prevalence** (%)	95% Confidence Interval
< 3 months	3	0	0	0 – 70.8
3-6 months	5	1	20	0.5 – 71.6
7-9 months	3	1	33.33	0.8 – 90.6
10-12 months	4	0	0	0 – 60.2
Total	**15**	**2**	**13.3**	**1.7 – 40.5**

Sex wise distribution of *Giardia lamblia* infection in children and calves is shown in Table 4 and 5. Males were at higher risk for developing Giardia infection compared to females both in children and calves.

Table 4. Sex specific distribution of *Giardia lamblia* infection in hospitalized children with diarrhea in ICMH, Dhaka

Sex	No. tested	No. positive	**Prevalence** (%)	Odds Ratio	95% Confidence Interval
Female	97	2	2.1	Reference	Reference
Male	169	8	4.7	2.21	0.46 - 10.8
Total	**266**	**10**	**3.8**	**-**	**1.8 – 6.8**

Table 5. Sex specific distribution of *Giardia lamblia* infection in calves

Sex	No. tested	No. positive	**Prevalence** (%)	95% Confidence Interval
Male	7	1	14.3	0.4 – 57.8
Female	8	1	12.5	0.3 – 52.7
Total	**15**	**2**	**13.3**	**1.7 – 40.5**

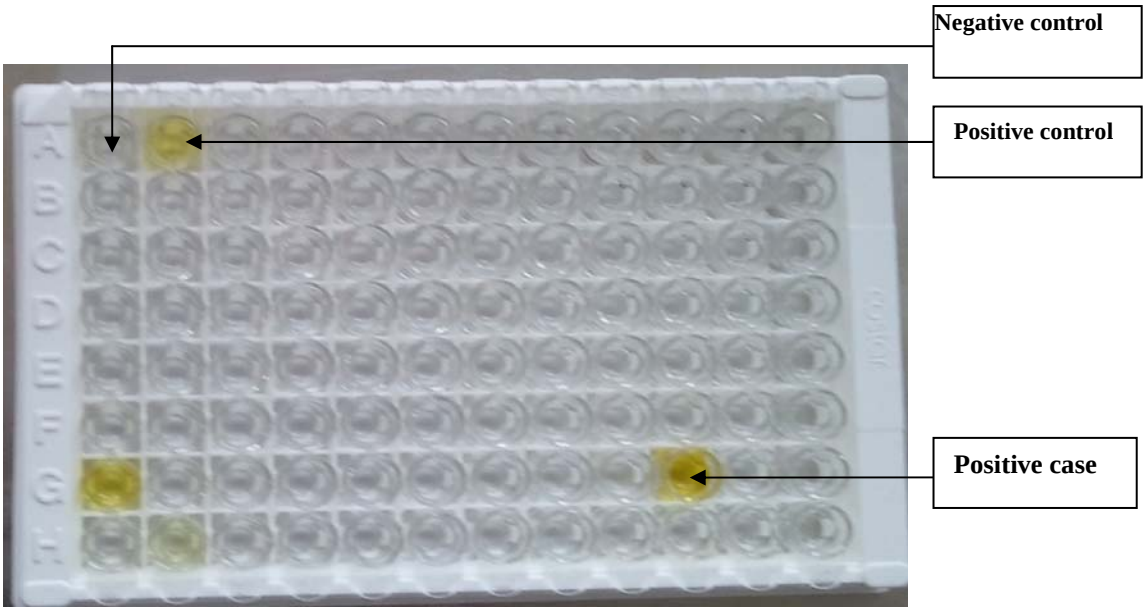

Figure 1. Microwells containing anti-Giardia antibodies - 96 test wells in a test strip Holder

In the present study, the prevalence of *Giardia lamblia* infection was 3.8% in children. In contrast to this study, Haque *et al.* (2003) reported higher prevalence (11%) in children living in the urban slum areas. These two studies have different settings, one based on hospitalized diarrheic patients and the other on slum dwellers. Overcrowding, lack of personal hygiene and sanitary conditions of the area, contaminated drinking water, may be responsible for higher prevalence of *Giardia lamblia* infection in slum areas. However, the prevalence of this study was in accordance with previous studies (Ngan *et al.*, 1992; Verle *et al.*, 2003).

Bjorkman *et al.* (2003) estimated 3.1% (1.5%–5.3%) *Giardia lamblia* infection in children by immunological assay (ELISA) is in agreement with the present findings. However, Alam *et al.* (2011) and Guillermo *et al.* (2011) found contrary to the present study though same diagnostic technique has been used. It may be due to different study area, sample size, age group etc. As microscopical examination has some disadvantage (time consuming and sensitivity could be affected by intermittent excretion of intestinal parasites), ELISA is more preferable for rapid diagnosis of large size sample.

The prevalence of *Giardia lamblia* infection in children was highest (8.7%) between 24 to 60 months of age was similar to the previous studies (Haque *et al.*, 2003). The possible reasons for this age dependent pattern are probably related to children habits (e.g. sharing things among themselves, putting objects into the mouth). Another reason for higher infections in children may also be related to the lack of effective immunity.

Studies in India and Nigeria have shown that boys were more likely to be infected with the *Giardia lamblia* than girls (Ranjan *et al.*, 2004; Dewivedi *et al.*, 2007) which is similar to our findings. Male was associated with a significantly higher relative risk for the first episode of symptomatic giardiasis.

Giardia has the potential to cause clinical disease in calves and to be transmitted to other animal species and humans, detection of *Giardia lamblia* in calves therefore may be of great public health significance, as humans and animals share sometimes the same premises in rural settings. About 13.3% calves were found to be infected with *Giardia lamblia* in our study. Similar observations were also made by others (Huetink *et al.*, 2001; Appelbee *et al.*, 2003). However, higher prevalence of *Giardia lamblia* infection was reported by other studies (Gow *et al.*, 2006; Geurden *et al.*, 2008). This difference may be due to sample size and diagnostic techniques.

This is the first study about the prevalence of *Giardia lamblia* infection in calves in Bangladesh by Sandwich-ELISA method. A greater awareness of parasitic contamination of the environment and its impact on health has precipitated the development of better detection methods for *Giardia lamblia*. The pathological study and seasonal dynamics on prevalence of *Giardia lamblia* is not yet studied which would be more effective in treatment and control measure against the *Giardia lamblia* infection in humans and calves, so a large scale study is needed in order to get accurate estimation of both in humans and animals for implementing effective control measures of *Giradia lamblia* in future.

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