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First Genotype Characterization of *Giardia intestinalis* Assemblage E from Goat Kids in Bangladesh

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Abstract

Giardiasis is an important protozoan disease caused by *Giardia* and manifested as life-threatening diarrhea in animals and humans worldwide. No study has performed to see zoonotic epidemiology and diversity of *Giardia* in animals in Bangladesh. Hence, there is a room to characterize *Giardia* protozoan parasite in animals to find out their roles as a source of human infection. To address this hurdle, fecal samples of 100 goat kids were genotyped by nested PCR amplification of *β-giardin* gene fragment followed by sequencing and analysis. The total prevalence of *Giardia* in goat kids was 3% (3/100) and the infection is more widespread in younger, Black Bengal breed and female goat kids. Further analysis of *β-giardin* gene locus has shown and clustered the *Giardia* in Assemblage E rather than Assemblages A and B. It suggests the low zoonotic transmission frequency from the goat kids and has least epidemiological significance to humans. Further study on this field is prerequisite in terms of broad geographical areas, age groups, sex and evaluates zoonotic significance along with genetic diversity in other host species as well.

Keywords: Genotypes; *Giardia*, Giardiasis; Nested PCR; Phylogenetic tree; Sequencing

Introduction

Giardia is a single celled protozoan parasite and frequently causes diarrheal diseases among many animals like domestic, cats, dogs, wild lives and the phenomenon is called giardiasis [1]. Hence, it becomes hardest currency to identify giardiasis with sings, symptoms and sensitive molecular characterization tools. Many symptoms have associated to cluster the disease e.g. diarrhea, consistent weight loss, fail to develop vigorously and sometimes death [2]. Immune-suppressed individuals allowing the parasite to cause and spread infections more easily and often become most vulnerable hosts [3] even those with limited pathogenicity. Robertson has showed more than 30% young ruminants' infections caused by *G. intestinalis* might be a major public health concern in both nation and society. It is because zoonotic transmission may occur from infected animals to humans through many ways like contamination of aquatic flora and fauna, environment and so on, and directly enter into human food chains [4]. Hence, giardiasis caused by *G. intestinalis* is considered as a zoonotic disease, since it contaminates and infects zoonatic living systems.

G. intestinalis is currently classified into eight Assemblages (A to H) on the basis of molecular characterization [5]. Generally Assemblage E which is called hoofed Assemblage is considered as the predominant gynotype of *G. intestinalis* in calves [6-8], sheep and goats [9-11] but Assemblage A and B have also been observed in these two ruminant species [7,8,11,12]. Currently, there is considerable evidence on the epidemiology of *Giardia* of ruminant [9,13], especially goats [10-12] in some countries, but more studies are necessary to disclose their prevalence in zoonatic transmission to human.

In Asia, Bangladesh has the highest population of goat and has 20.75 million goats at present [14]. The prevalence of *Giardia* infection in human was studied previously in Bangladesh [15]. However, there is no study has put forth to evaluate involvement of goats in the *G. duodenalis* epidemiology and transmission mechanism for humans. The goats could commonly infected by various parasites due to the agro-ecological and geo-climatic conditions, which are highly favorable for *G. duodenalis* growth and multiplication [16]. Therefore, the present study has focused on molecular characterization of *Giardia* in goat kids to elucidate their possible zoonotic hazards in this region, according to different age, breeds (Black Bengal and Jamunapari) and sex. In addition, we have compared the evolutionary relationship with other *Giardia* infection not only in goats but also in other animals in different countries. It will help to understand actual epidemiological picture in terms of zoonotic potentials of *Giardia* infection in humans among different countries people.

Materials and Methods

Collection of fecal samples

A total of 100 fresh fecal samples were collected from goat kids [1-6 months old] from a local veterinary clinic in Chittagong, Bangladesh. All were affected with diarrhea when admitted to the clinic. Feces were collected directly from the rectum of each goat into a plastic specimen cup followed by immediately capped, labeled properly. Feces were preserved in -20°C freezer until DNA extraction was carried out.

DNA extraction

Genomic DNA was extracted from fecal samples, using the QIAamps stool mini kit [Qiagen] according to manufacturer's instructions and stored at -20°C. Extracted DNA concentration was

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measured by Thermo scientific Nano drop 2000 spectrophotometer [A339, USA].

Nested PCR analysis

Nested PCR analysis was performed to identify *β-giardin* gene locus in *G. intestinalis*. Forward and reverse primers namely G7 and G759 have been used in primary PCR reaction to amplify about 753 bp target DNA fragments [17]. In addition, secondary PCR reaction has performed by using forward primer G7n and reverse primer G759n in order to amplify about 511 bp target DNA fragments [18]. All the reaction mixtures and cycling conditions were same as previously published standard protocols [18]. In each experiment, positive control was carried out as standard *Giardia* genomic DNA along with sample run without DNA template as negative control element. A PCR thermal cycler [Applied Biosystem 2720 Thermal cycler®] was used in both primary and secondary PCR reaction cycles and the products were analyzed by 1.5% agarose gel electrophoresis.

DNA sequencing and submission

The PCR products of representative strains from each restriction group were purified with the PCR Clean-Up kit [Promega®, USA] and were sequenced on an ABI sequencer [ABI Prism, 3130, USA]. After sequencing of the representative nested PCR products, the quality of the sequence was carefully assessed manually based on the corresponding electro-chromatogram and have submitted in GenBank NCBI database [19].

The final nucleotide sequence data reported in this paper are available in the GenBank under the accession numbers JX122074, JX122075, and JX122076**.**

Determination of specific genotypes and sub genotypes

Specific Assemblage of Giardia of positive isolates was determined by comparing the sequences using the Basic Local Alignment Search Tool. The variations of nucleotide position at different points were revealed using ClastalW2 multiple sequence alignment programme to classify genotype into subgroups.

Construction of phylogenetic tree

To compare nucleotide variability and genetic relationship of *β-giardin* gene sequences of goat kids in Bangladesh [Genbank: JX122074, JX122075 and JX12076] with other infected animals in different countries, a phylogenetic tree was constructed. During this study top 23 BLAST hits of significant E-value [0.00] of *β-giardin* gene sequences of Genbank accession no. JX122074, JX122075 and JX12076, respectively were retrieved. Regenerative sequences of hits have eliminated in order to get non-redundant *β-giardin* gene sequences. The sequences were retrieved from GenBank database [19] and were initially aligned under default conditions by using MUSCLE program [20]. Furthermore, identical conserved regions of sequences of scored in the alignment was used to generate the tree. The sequence alignment was performed under default conditions and the tree was constructed by the neighbor-joining method [21]. The poorly aligned sequence and also the internal gaps residue were taken off from the alignments to make a precise evolutionary tree by using the Jalview program [22]. Finally, the phylogenetic tree of β-giardin gene sequences was constructed by using [ClustalW2-Phylogeny] program [23].

Results

The *β-giardin* gene was amplified from individual genomic DNA samples by nested PCR assay during this study. The PCR analysis of 100 isolates resulted only 3% [n=100] have given positive bands. This was identified by observing the band with respect to marker on 1.5% agarose gel on the basis of 753bp in primary PCR and 511bp in secondary PCR, respectively. Figure 1 shows the nested PCR result, where positive isolates of *Giardia* have visualized as distinct bands.

Table 1 presents the detail molecular findings of present study, where positive isolates were classified according to age, breed and sex. It was observed that among 3 positive isolates, 2 were female and 1 was male. In addition, the age of goat kids sampled during this study was between 1 and 6 months. Two goat kids were 2 months old and remaining one was of 1 month of age.

The secondary PCR product of 3 representative strains were sequenced and deposited in GenBank database under following accession numbers Genbank: JX122074 [isolate N76], Genbank: JX122075 [isolate N90] and Genbank: JX122076 [isolate N94], respectively. The three sequences were belongs to Assemblage E, which have been confirmed by nucleotide BLAST available at NCBI databases. Furthermore, we have classified this single Assemblage E into two sub genotypes [E1 and E2] on the basis of similarities and identities of β-giardin gene sequences by using multiple sequence alignment. The sequences [Genbank: JX122074, JX122075] have shown similar but the sequence [Genbank: JX122076] has shown genetic polymorphism with one to four base variations at 14 nucleotide sites at positions 127, 131, 133, 135, 151, 164, 169, 172, 173, 198, 199, 201, 223 and 300 [data not shown]. Genbank: JX122074 and JX122075 have shown subtype E1 and have 100% similarity with conserved identical nucleotides of the positions, whereas Genbank: JX122076 has considered as subtype E2,

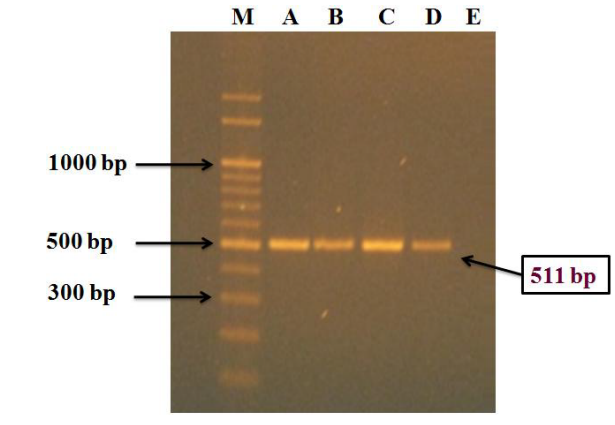

Figure 1: Electrophoretic [1.5% Agarose] separation of β-giardin gene, Lane M, ladder [Marker]; lane A ,positive control; lane B, isolate N76; lane C, isolate N90 and lane D, isolate N94 and E, negative control.

Total no. of samples	Age [Month]						Breed		Sex	
	1	2	3	4	5	6	BB*	JP**	Female	Male
100	0	2[2%]	1[1%]	0	0	0	2[2%]	1[1%]	2[2%]	1[1%]

BB-Black Bengal; JP- Jamunapari

Table 1: Molecular characterization of β-giardin gene of *Giardia* spp. in the goat kids of present study.

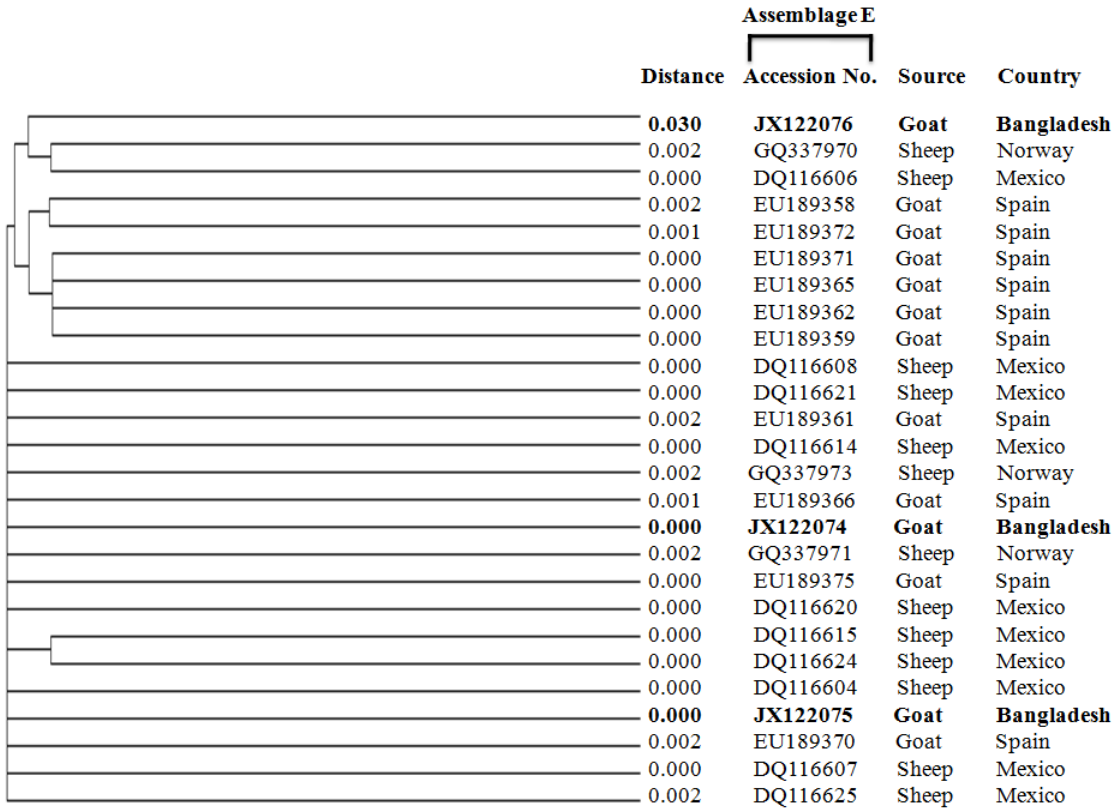

Figure 2: The genetic relationship of *Giardia intestinalis inferred from β-giardin gene sequences following analysis using* neighbor-joining method of ClustalW2-Phylogeny.

and shown 97% similarity with other standard sequences of infected animals from different countries available with following Genbank: EU189375, EU189366, EU189361, DQ116625, DQ116620, DQ116614, DQ116608 and DQ1166O7. The sequence Genbank: JX122076 has shown variations at 14 nucleotide sites due to its genetic mutations and diversity during evolution.

Figure 2 shows dendogram tree, depicted an intragenotypic variation of *β-giardin* gene sequences Genbank: JX122074, JX122075 and JX122076. The degree of variability noticed among the goat isolates in *β-giardin* gene sequences as they might have different orthologus origin, respectively. Isolate N76 [Genbank: JX122074] from goat is closely related with isolates [Genbank: EU189366] and [Genbank: GQ337971] from goat and sheep, respectively. And isolate N90 [Genbank: JX122075] has closely genetically related with isolates Genbank: DQ116604 and Genbank: EU189370 from sheep and goat, respectively. But, isolate N94 [Genbank: JX122076] has got special attention, as the sequence distantly related from other sequences. All sequences might be descended from this isolate N94 [Genbank: JX122076] as it has most genetic distance 0.030 among all isolates. However, pair wise genetic distances among all isolates were ranged from 0.000 to 0.030 with an average mean value 0.001, which indicates the proximity of all isolates during evolution

Discussion

Samples were used during this study, received from goat kids reared in Chittagong metropolitan areas. Molecular analysis revealed that the prevalence of *Giardia* was only 3% in goat kids and represent sporadic cases occurring in this part of the country (Figure 1). However in goat, giardiasis has been reported to occur in different parts of the world and prevalence rate was also different in the ranged between 6.8% and 89.2%. For example, the occurrence of giardiasis was 6.8% in Malaysia [11], 89.2% in Spain [24], 25.5% in Belgium [25], 42.2% in Spin [10], 12% in Maryland, USA [9], 19.8% in Spain [12] and 14.3% in Brazil [26]. It can be postulated that the comparative high rate of infection in these countries could be due to high level of environmental contamination which is usually very different in Bangladesh, since we observed the rate only 3%. Most small ruminants like sheep and goat in Bangladesh have little access to pasture and usually they are reared in intensive or semi-intensive method where there might be less possibility for the young animals to be infected by the contaminated cysts.

Age resistance is always an important issue in any parasitic disease. We have examined whether there is any correlation between age of goat kids and the prevalence of giardiasis. Among three positive isolates, two goat kids were 2 months old and remaining one was of 1 month of age (Table 1). So, young animals might be most vulnerable hosts to parasite infection. It might be due to less ability to induce immune system, since young infants have low immune complements and antibodies. This result has fully supported by other published studies [10,12]. In case of cattle, it was also observed that infection of *Giardia* was highly prevalent in younger calves than in adult cattle [27].

To determine the relationship between goat's breed and *Giardia* infection, we have collected samples from two different local goat breeds namely Jamunapari and Black Bengal goat kids. We have found

Jamunapari are less vulnerable to giardiasis compared to the Black Bengal goat kids (Table 1). Although this hypothesis with little sample size, further large scale sampling can be more informative to establish this hypothesis. This can initiate new thoughts on breed-associated immunologic factors and future research can answer if there is any such probability or not.

Prevalence of giardiasis might occur with sex-specific preference in goat kids. We have found two female and one male infected goat kids, who have shown signs and symptoms of giardiasis (Table 1). This finding is consistent with previously published study [28]. One would assume that there is significant level of sex-specific variation in illness among different groups of kids. Though prevalence of giardiasis according to male and female goats are rarely studied, in case of human it was found that infection rate of *Giardia* in male and female were 1.5% and 2.18%, respectively [29]. In one study involving dog, it was reported that female [28%] were more susceptible to giardiasis than male dogs [10.90%] [28]. Further study can highlight if there is any factors responsible for this sex-specific vulnerability of animals as well as human suffering from giardiasis as well.

Although in Bangladesh, assemblages A and B have associated with human infections [15], assemblage E could indirectly participated to stimulate the zoonatic epidemiology. Assemblage E is frequently present in many animal species have observed in different countries published elsewhere [6-12], whereas recent study has suggested assemblages A and B to be present in ruminants [11]. This cross behavior indicates the susceptibility of infection of assemblage E in human or vice versa. However, the assemblage E is not considered to be a strong zoonotic assemblage and therefore, giardiasis in goat could be of low epidemiological significance for possible zoonotic transmission in absence of others. The rate of infection in other small ruminants like sheep and lambs could be useful to understand the distribution of different genotypes and assemblages in any specific area. This will ultimately help to assess environmental contamination level and associated risks of *Giardia* in a particular community.

There are few studies have described the intra-genotypic diversity of Assemblage E in goats [10,25]. We have classified Assemblage E into two subtypes namely [E1 and E2] on the basis of nucleotide polymorphism sites present. E1 subtype includes two isolates N76 [Genbank: JX122074] and N90 [Genbank: JX122075] and E2 has isolate N94 [Genbank: JX122076]. Moreover, the transmission dynamics and public health significance of the subtypes E2 need to be studied extensively for a better understanding of their unique genetic makeup. Figure 2 shows genetic diversity and/or variability of isolates N76 [Genbank: JX122074], N90 [Genbank: JX122075] and isolate N94 [Genbank: JX122076]. It presents the evolutionary closed relationship with other orthologus genes from goats and sheep in Spain, Mexico and Norway. But the rate of polymorphism was not too high in terms of lower genetic distances observed. Gene flow might occur during evolution of *Giardia* and might causes different haplotypes in animals from different countries. Therefore, the variable sequence of *β-giardin* gene in same Assemblage of *Giardia* spp. could be a better characterizing tool to discriminate these species from each other. Such genotyping could play a vital role to elucidate the pathogenicity, epidemiology and ultimately the mechanisms of host-parasite interactions.

Conclusion

It was observed that younger, Black Bengal breed and female goat kids are more vulnerable than older, Jamunapari breed and male goat kids, respectively. Occurrence of Assemblage E during this study suggests that zoonotic transmission of *Giardia* spp. from goat kids could be of low epidemiological significance in absence of Assemblages A and B. However, further study on epidemiological investigation of humans, domestic and wild animals as well as water catchment areas and drinking water sources is necessary to acquire better information about the prevalence, host affiliations and geographical distributions of the different Assemblages of *G. intestinalis* in animals from different countries.

Competing Interest

The authors declared that they have no competing interests.

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References

1. Adam RD (2000) The Giardia lamblia genome. Int J Parasitol 30: 475-484.
2. Aloisio F, Filippini G, Antenucci P, Lepri E, Pezzotti G, et al. (2006) Severe weight loss in lambs infected with Giardia duodenalis assemblage B. Vet Parasitol 142: 154-158.
3. Adam RD (2001) Biology of Giardia lamblia. Clin Microbiol Rev 14: 447-475.
4. Farizawati S, Lim YA, Ahmad RA, Fatimah CT, Siti-Nor Y (2005) Contribution of cattle farms towards river contamination with Giardia cysts and Cryptosporidium oocysts in Sungai Langat Basin. Trop Biomed 22: 89-98.
5. Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. Clin Microbiol Rev 24: 110-140.
6. O'Handley RM, Olson ME, Fraser D, Adams P, Thompson RC (2000) Prevalence and genotypic characterisation of Giardia in dairy calves from Western Australia and Western Canada. Vet Parasitol 90: 193-200.
7. Trout JM, Santín M, Greiner E, Fayer R (2005) Prevalence and genotypes of Giardia duodenalis in post-weaned dairy calves. Vet Parasitol 130: 177-183.
8. Trout JM, Santín M, Fayer R (2007) Prevalence of Giardia duodenalis genotypes in adult dairy cows. Vet Parasitol 147: 205-209.
9. Santín M, Trout JM, Fayer R (2007) Prevalence and molecular characterization of Cryptosporidium and Giardia species and genotypes in sheep in Maryland. Vet Parasitol 146: 17-24.
10. Ruiz A, Foronda P, González JF, Guedes A, Abreu-Acosta N, et al. (2008) Occurrence and genotype characterization of Giardia duodenalis in goat kids from the Canary Islands, Spain. Vet Parasitol 154: 137-141.
11. Lim YA, Mahdy MA, Tan TK, Goh XT, Jex AR, et al. (2013) First molecular characterization of Giardia duodenalis from goats in Malaysia. Mol Cell Probes 27: 28-31.
12. Castro-Hermida JA, Almeida A, González-Warleta M, Correia da Costa JM, Rumbo-Lorenzo C, et al. (2007) Occurrence of Cryptosporidium parvum and Giardia duodenalis in healthy adult domestic ruminants. Parasitol Res 101: 1443-1448.
13. Trout JM, Santín M, Greiner EC, Fayer R (2006) Prevalence and genotypes of Giardia duodenalis in 1-2 year old dairy cattle. Vet Parasitol 140: 217-222.
14. Department of Live stock Services (DLS) (2007) Livestock statistics of Bangladesh.
15. Haque R, Roy S, Kabir M, Stroup SE, Mondal D, et al. (2005) Giardia assemblage A infection and diarrhea in Bangladesh. J Infect Dis 192: 2171-2173.
16. Haq S, Shaikh H (1968) A Survey of Helminth parasiting the gastro-intestinal tracts of goats and sheep in East Pakistan. Pak J Vet Sci. 2: 54-62.
17. Caccio SM, De Giacomo M, Pozio E (2002) Sequence analysis of the ß-giardin gene and development of a polymerase chain reaction–restriction fragment

length polymorphism assay to genotype Giardia duodenalis cysts from human faecal samples. Int J Parasitol 32: 1023-1030.

18. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, et al. (2005) Genetic heterogeneity at the beta-giardin locus among human and animal isolates of Giardiaduodenalis and identification of potentially zoonotic subgenotypes. Int J Parasitol 35: 207-213.
19. Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, et al. (2012) GenBank. Nucleic Acids Res 40: D48-53.
20. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792-1797.
21. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.
22. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ (2009) Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics 25: 1189-1191.
23. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.
24. Gómez-Muñoz MT, Cámara-Badenes C, Martínez-Herrero Mdel C, Dea-Ayuela MA, Pérez-Gracia MT, et al. (2012) Multilocus genotyping of Giardia duodenalis in lambs from Spain reveals a high heterogeneity. Res Vet Sci 93: 836-842.
25. Geurden T, Geldhof P, Levecke B, Martens C, Berkvens D, et al. (2008) Mixed Giardia duodenalis assemblage A and E infections in calves. Int J Parasitol 38: 259-264.
26. Bomfim TC, Huber F, Gomes RS, Alves LL (2005) Natural infection by Giardia sp. and Cryptosporidium sp. in dairy goats, associated with possible risk factors of the studied properties. Vet Parasitol 134: 9-13.
27. Khan SM, Debnath C, Pramanik AK, Xiao L, Nozaki T, et al. (2011) Molecular evidence for zoonotic transmission of Giardia duodenalis among dairy farm workers in West Bengal, India. Vet Parasitol 178: 342-345.
28. Biancardi P, Papini R, Giuliani G, Cardini G (2004) Prevalence of Giardia antigen in stool samples from dogs and cats. RevMedVet - Revue de Medecine Veterinaire 155: 417-421.
29. Ibrahim AQ (2012) Prevalence of Entamoeba histolytica and Giardia lamblia in Children in Kadhmiyah Hospital. The Iraqi J Vet Med 36: 32-36.