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Research Paper

DETECTION OF GIARDIA LAMBLIA ASSEMBLAGE E USING NESTED PCR AMONG CHILDREN IN SOHAG CITY, EGYPT

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The purpose of this study was the detection *Giardia lamblia* (*G.lamblia*) assemblage E (host specific for animals) in stool samples of children suffering from gastrointestinal disturbance admitted to Sohag University hospital, Sohag, Egypt. Stool samples were microscopically examined by formalin ethyl acetate sedimentation test for observation of *Giardia species* (trophozoite or cyst). Then nested PCR was used to detect *G.lamblia* assemblage E through triosephosphate isomerase gene (Tpi gene) amplification from positive stool samples. Risk factors related to infection were determined based on a data collected from each child parents through a standard form. The results revealed that 12 (25.5%) out of 47 of the examined children were positive for *G.lamblia* assemblage E. Referring to risk factors associated with infection, contact with animals, raised in rural area and playing outdoor were significantly correlated with infection. This study aimed to detect the role of animals in transmission of *G.lamblia* assemblage E to children and spotlights on the risk factors related to infection.

Keywords: G.lamblia, Tpi gene, Nested PCR, Assemblage E

INTRODUCTION

G.lamblia is the most common intestinal parasite in human, animals and birds all over the world. In low income countries, *Giardia* prevalence in children was increased up to 30% mostly in those younger than ten years old (Younas *et al.*, 2008). The clinical symptoms ranged from diarrhea, abdominal discomfort, loss of weight, chronic malnutrition to poor perceptual function with prolonged infection (Halliez and Buret, 2013).

G.lamblia (synonyms: *G.intestinalis and G.duodenalis*) genetically includes eight assemblages (A, B, C, D, E, F, G and H). Assemblages A and B mainly affect human and other livestock and companion animals such as cattle, pig, sheep, horse and non human primates, so, considered as Zoonotic assemblages (Durigan *et al.*, 2014), while C and D infect dogs, E in livestock animals, F for cats, G in rats and assemblage H was identified in

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marine vertebrates such as seals (Adam, 2001). Although assemblage E recognized as host specific for animals, recent researches reported that animal assemblages (from C to H) were detected in human samples (Štrkolcová *et al.*, 2015). This indicated that animal types of *Giardia species* can cross other species barrier and transmitted to humans. Therefore, during epidemiological investigation and risk factor determination, it is important to determine *Giardia* assemblages (Betancourt and Rose, 2004) by the molecular detection of Tpi gene as it is helpful in the detection and differentiation between *Giardia* assemblages (Sulaiman *et al.*, 2003). This study spotlights on the role of animals in transmission of *G.lamblia* assemblage E to human especially children using nested PCR for detection of animal host specific *G.lamblia* assemblage E through amplification of triosephosphate isomerase gene (Tpi) among children stool samples and determine the risk factors related to infection.

MATERIALS AND METHODS

Stool Samples and Data Collection

A total of 47 stool samples were collected from children with gastrointestinal disturbance their age ranges from one to 11 years admitted to Sohag University Hospital, Sohag, Egypt. Data were collected from parents of children through a standard form including information about child age, gender, locality, animal contact, water supply and playing outdoors. Parents were informed about the aim of the study and the data were collected after their consent. Stool samples were collected in sterile cups and taken to the laboratory for microscopical examination by formalin ethyl acetate sedimentation technique (Levine and Estevez, 1983). Positive samples for *Giardia species* (trophozoite or cyst) were confirmed by nested PCR for detection of *G.lamblia* assemblage E.

DNA Extraction and Nested PCR

DNA was extracted from stool samples using QIAamp DNA stool Mini Kit (Qiagen, Germany) based on the kit instructions. Two reactions were performed for nested PCR, for the primary PCR, a PCR product of 605 bp was amplified for Tpi gene by using primers AL3543 [52-AAATIATGCCTGCTCGTCG-32] and AL3546 [52-CAAACCTTITCCGCAAACC-32]. The reaction of PCR for Tpi gene was done based on (Sulaiman *et al.,* 2003). For the secondary PCR, a fragment of 388 bp was amplified by using primers [5'-CCCCTTCTGCCGTACATTTAT-3'] and [5'-GGCTCG TAAGCAATAACGACTT-3'] to detect *G.lamblia* assemblage E. The primary PCR product was used as a template DNA for the secondary reaction. The reaction for assemblage E was done according to (Geurden *et al.,* 2008) with changes in the cycling condition as 5 min at 95 ºC , then 35 cycles of 30 sec at 94 ºC, 30 sec at 58 ºC, 20 sec at 72 ºC and final extension for 5 min at 72 ºC using thermal cycler from (Bio-Rad, USA). Electrophoresis for PCR product was done by stained agarose gel (1.5%) with ethidium bromide and photographed with UV light transilluminator (Biometra).

Statistical Analysis

SPSS version 14 (SPSS, Inc., Chicago, IL, USA) was used for analyzing of data. P value < 0.05 is considered significant.

RESULTS

Among 47 stool samples collected from children suffering from gastrointestinal disturbance, *G.lamblia* assemblage E was detected in 12 (25.5%) samples by nested PCR (Table 1). Most of the examined children were in age group from

Table 1: Occurrence of G.lamblia Assemblage E Among Children Stool Samples

Children Samples	No. of Examined Children	Microscopy (*G. lamblia*)		Nested PCR (*G. lamblia* Sssemblage E)	
		No	%	No	%
Stool	47	16	34	12	25.5

Table 2: Risk Factors Related to G.lamblia Assemblage E Infection Among Children

Risk Factors	No. of Examined Children N/47		*G.lamblia* Assemblage E N/12		P value
	No	%	No	%	
Age[a]					0.847
1- 3 years	18	38.3	4	33.3	
4- 7 years	17	36.2	6	50	
8- 11 years	12	25.5	2	16.7	
Gender[a]					0.813
Male	26	55.3	7	58.3	
Female	21	44.7	5	41.7	
Locality[b]					0.05
Rural	18	38.3	8	66.7	
Semi urban	15	31.9	3	25	
Urban	14	29.8	1	8.3	
Animal contact[b]					0.01
Yes*	24	51.1	10	83.3	
Cattle	11	23.4	6	50	
Dogs	9	19.1	5	41.7	
Cats	7	14.9	6	50	
Chicken	15	31.9	7	58.3	
Rabbits	5	10.6	2	16.7	
No	23	48.9	2	16.7	
Water supply[a]					0.412
Tape water	32	68.1	7	58.3	
Dug well with hand pump	15	31.9	5	41.7	
Playing outdoor[b]					0.05
Yes	25	53.2	10	83.3	
No	22	46.8	2	16.7	

Note: * Some children exposed to more than one animal species. [a] Non-significant factors; [b] Significant factors.

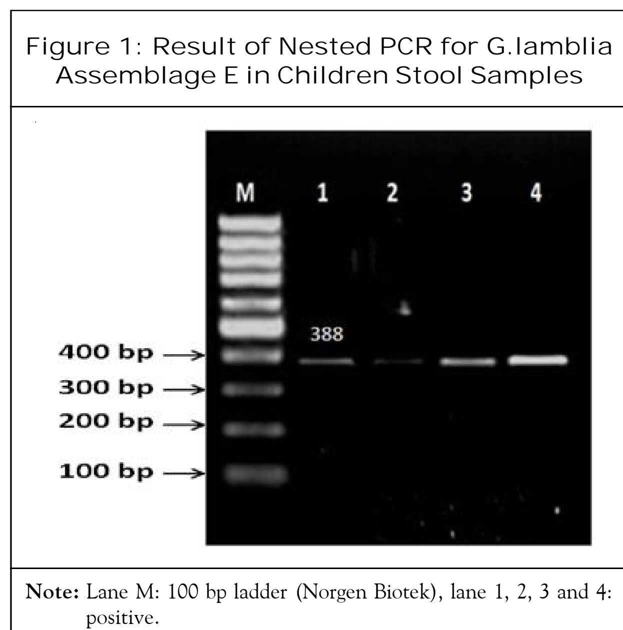
one to three years (38.3%), 26 males (55.3%), 18 (38.3%) live in rural area, 24 (51.1%) in contact with animals and 25 (53.2%) playing outdoors (Table 2).

DISCUSSION

Table 1 illustrated that 12 (25.5%) out of 47 stool samples collected from children were positive for *G.lamblia* assemblage E. This result is higher than that obtained by Helmy *et al.* (2014) and Foronda *et al.* (2008) and opposite to Amer (2013), Ehsan *et al.* (2015) and Fahmy *et al.* (2015) who cannot detected *G. lamblia* assemblage E in human samples. The presence of animal host specific assemblage in stool samples of children revealed that assemblage E have the ability to jump and cross barrier species to infect humans and other animals.

Using nested PCR for amplification of Tpi gene assemblages is highly specific for assemblage E detection even with low number of *Giardia* cyst (Geurden *et al.,* 2008). Nested PCR was used to detect *G.lamblia* assemblage E in children stool samples (Figure 1). Detection of *G.lamblia* assemblages in human samples give more information about the epidemiology of *Giardia* as source of infection, prevalence, risk factors related to infection and to understand the Zoonotic potential of *G.lamblia* infection.

Figure 1: Result of Nested PCR for G.lamblia Assemblage E in Children Stool Samples



Note: Lane M: 100 bp ladder (Norgen Biotek), lane 1, 2, 3 and 4: positive.

The characteristics of the examined children revealed that children in the age group from four to seven years reported the highest infection rate (50%) followed by one to three years (33.3%) and eight to 11 years (16.7%) (Table 2). This explained by that child in small age unaware about the hygienic measures, have the habit of hand to mouth and presumably with immature immune system (Mateo *et al.*, 2014).

Referring to risk factors associated with infection; localities, contact with animals and playing outdoors were significantly correlated with G.*lamblia* assemblage E infection (P > 0.05, 0.01 and 0.05 respectively). Therefore, direct or indirect contact with animals is the primary risk factor for assemblage E infection. According to the obtained data, 10 (83.3%) out of 12 infected children were in contact with animals either at home or during playing outdoors. In addition, children raised in rural area were more exposed to infection than those in urban area as eight (66.7%) out of 12 children live in rural area in which animals mostly reared such as dogs, cattle, cats and poultry. People in rural area left their animals to roam freely outdoors in non paved streets and contaminate the water and soil with their feces. So, animal feces considered a main source of *Giardia* infection. Children get infected by handling of infected animals, eating unwashed food as vegetables and fruits. Also, drinking of contaminated water especially during their playing and swimming in water streams particularly in hot weather.

Most of the infected children were exposed to different animal species other than livestock animals as dogs (41.7%), cats (50%) and rabbits (16.7%). Although *G. lamblia* assemblage E was mainly host specific for animals (Adam, 2001), other researches was detect assemblage E in

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rabbit (Qi *et at.*, 2015), cat, non human primates and dogs (Johnston *et al.*, 2010). Therefore mixed rearing of different animal species may increase the risk of cross species transmission of assemblage E from livestock animals to other species of animals and human (Leveckea *et al.*, 2016). Thus, further studies are needed to detect the prevalence of *G.lamblia* especially in rural areas and detriment the risk factors correlated with infection to set a control strategy against *G.lamblia* infection.

CONCLUSI ON

G.lamblia assemblage E has the ability to cross animal species barrier and infect children. Infection was more prevalent in rural environment with poor hygienic measures, unrestricted animal movement and mixed animal rearing. Epidemiological studies and determination of infection risk factor is required to prevent *Giardia* infection through an effective control plan.

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