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Giardia lamblia in household persons and buffalo calves; prevalence, molecular identification and associated risk factors

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Abstract

A total of 195 faecal samples comprising (100 from buffalo calves and 95 from household persons) were parasitologically examined for the presence of *Giardia lamblia* using direct smear method and concentration techniques. The overall prevalence among household persons was 17.9% with a higher infection rate 24.4% in children compared to 12% in adults. Moreover, 28% of diarrhoeic versus 20% of non- diarrhoeic children and 15.4% of diarrhoeic versus 8.3% of non- diarrhoeic adults were shedding *Giardia* cysts. The overall infection rate in calves was 25% with a significant higher rate 30% ($P < 0.05$) among diarrhoeic versus 17.5% in non-diarrhoeic ones. Multivariate logistic regression analysis retained not washing hands after contact with calves and their manure as a potential significant risk factor strongly associated with the infection (OR= 40.67; CI: 3.89-424.85; P=0.002). Based on nested-PCR results, 292-bp fragment of SSUrRNA gene of *G. lamblia* was amplified in faecal samples from household persons and calves. Our results emphasize the role of buffalo calves in zoonotic transmission of *G. lamblia* to persons living in the same households.

Key Words: *Giardia lamblia,* household persons, buffalo calves, PCR

Introduction

Giardia is a protozoan parasite mainly infects the gastrointestinal tract of humans and animals causing enteric disease. The genus of *Giardia* currently comprises six species, *G. lamblia (G. intestinalis* or *G. duodenalis*) was the only species recovered from humans and a wide range of mammalian hosts, considered as a zoonotic agent by the WHO[16)]. It is global in its distribution and adversely impact human health in both developed and developing countries. Transmission to humans can occur either through faecal–oral route following ingestion of contaminated food and water or direct contact with infected animals[10)]. Ruminants have been implicated as a major source for human infection based on the findings of many molecular epidemiological studies[17)]. For instance, epidemiological studies have assessed the importance of zoonotic transmission in the occurrence of human giardiasis and demonstrated that there is a strong link between human infection and direct or indirect contact with cattle particularly preweaned calves[12)]. In recent years, the zoonotic potential of *Giardia* is becoming clearer with the development of molecular

methods such as PCR based on amplification of the small subunit (SSU) rRNA gene for differentiation of *Giardia* species in animals' and humans' specimens[10]. Therefore, the present study was carried out to assess the zoonotic transmission of *Giardia* between persons and buffalo calves living in the same households through studying its prevalence in both sources and determination of the risk factors associated with human infection. Also, molecular identification of *Giardia* isolates was performed.

Materials and methods

Ethical approval:

The study was approved by Zagazig University ethical board. Informed consent was obtained from household persons to collect faecal samples from them and their owned calves.

Sampling:

One hundred faecal samples were collected from 1-4 months old buffalo calves between December, 2013 and September, 2014 from a rural village located in Sharkia Province, Egypt. Concurrently, stool samples were collected from only 95 household persons who live with the calves within the same households and gave informed consent. Simultaneously, with the collection of samples, adult household persons received comprehensive questionnaires that were filled with information relevant to household members (age, sex, exposure to calves, personal hygienic practices, health status) and their calves (age, sex and presence or absence of diarrheal symptoms at time of survey). Animals' and humans' faecal samples were labeled with the code on the corresponding questionnaire sheet, ice packed and transported immediately to Parasitology laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt for parasitological examination.

Parasitological examination:

The animals' and humans' faecal samples were examined macroscopically with naked eyes for color, consistency and microscopically by direct and concentration methods for the presence of Giardia trophozoite and cyst stages.

Direct smear method:

Direct smear method was performed according to Beaver *et al*.[3].

Concentration techniques

Formalin-ether sedimentation technique

The formalin-ether sedimentation technique was performed as described by Cheesbrough[5]. Pellets of the sediment were examined by emulsifying them in 1–2 drops of 1% lugol's iodine solution, then examined using a light microscope at 400 X magnification for detection of *Giardia* cysts and/or trophozoites. The samples with more than 15 *Giardia* cysts in averagely 10 microscopic fields were condensed and purified via Sheather's sugar floatation technique.

Cyst purification using Sheather's sugar floatation technique:

Two grams of feacal sample were emulsified in 10 ml of modified Sheather's sugar flotation solution (specific gravity= 1.27) and strained through a tea strainer into a 15-ml centrifuge tube. The tube was filled with floatation solution until a slight positive meniscus forms, centrifuged at 1200 r.p.m for 5min. The sucrose phase and upper layer of liquid were transferred using a pipette to another tube with two volumes of phosphate-buffered saline (PBS), centrifuged at 1500 r.p.m for 5 min for concentration of *Giardia* cysts[9] . The supernatant was discarded and the pellet was resuspended in PBS to a volume of 1ml and stored at -20 °C until used.

Cyst disruption and DNA extraction:

Fecal pellet of each purified sample was subjected to 3 successive cycles of boiling (100°C/2min) and freezing in liquid nitrogen for 2 min. followed by overnight incubation with proteinase K at 56°C[1]. DNA extraction was performed using the QIAamp DNA Stool Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations.

PCR amplification:

A nested PCR was performed to amplify a 292-bp fragment of SSUrRNA gene for confirmatory identification of *G. lamblia* using the following primers supplied from Metabion (Germany); GiaF: 5' -AAG TGT GGT GCA GAC GGA CTC-3', GiaR5'-CTG CTG CCG TCC TTG GAT GT-3' and RH115'-CAT CCG GTC GAT CCT GCC-3', RH4 5'-AGT CGA ACC CTG ATT CTC CGC CAG G-3'[2]. The PCR was carried out in standard mixtures of 25 μl containing 12.5 μl of EmeraldAmp Max PCR Master Mix (TAKARA, Japan), 1 μl of each primer of 20 pmol concentrations, 4.5 μl of water and 6 μl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler with the following amplification conditions; 35 amplification cycles (96 °C for 45 sec, 55 °C for 30 sec, 72 °C for 45 sec) followed by one cycle of 4 min at 72°C at the primary PCR, then 35 amplification cycles (96 °C for 45 sec, 59 °C for 30 sec, 72 °C for 30 sec) followed by one cycle of 4 min at 72 °C at the secondary PCR. The PCR products were analyzed by 2% agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 μl of the products were loaded in each gel slot. A 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed through computer software.

Statistical analysis:

Two-proportions Z- test was used to compare the occurrence proportions of both *G. lamblia* cyst and trophozoite among diarrhoeic and non-diarrhoeic household persons. Fisher exact test was used when normal approximation may be inaccurate for small samples. Univariate and multivariate logistic regression models were fitted to determine risk factors associated with Giardia infection in household persons. Univariate analysis was subjected to co linearity analysis determined by the Spearman's rank correlation test. Subsequently, multivariate logistic regression analysis using a backward-stepwise (conditional) selection model to select factors with a p value of less than 0.1 to be fitted into the multivariate logistic regression analysis. The statistical software packages (SPSS for Windows 21.0, Inc., Chicago, IL, USA) was used for data analysis and results are expressed as

Table 1. Age specific distribution of Giardia lamblia infection in household persons in relation to health status

Study subject	No. examined	*Giardia lamblia* cyst			*Giardia lamblia* trophozoite		
		No. (%)	Z-value	*P* value	No. (%)	Z- value	*P* value
Children							
Diarrhoeic	25	7(28)	0.63		1(4)		
Adults							
Diarrhoeic	26	4(15.4)			1(3.8)		
Non -diarrhoeic	24	2(8.3)	0.78	0.669	0(0)	1.02	1.000
Total	50	6(12)			1(2)		
Total	95	17(17.9)			2(2.1)		

Table 2. Occurrence of Giardia lamblia in fecal samples of diarrhoeic and non- diarrhoeic household buffalo calves.

Fecal state	No. examined	*Giardia lamblia* cyst			*Giardia lamblia* trophozoite		
		No. (%)	Z-value	*P* value	No. (%)	Z-value	*P* value
Diarrhoeic	60	18(30)	2.24	0.025 *	1(1.7)	1.01	1.000
Non -diarrhoeic	40	7(17.5)			0(0)		
Total	100	25(25)			1(1)		

* Represent statistically significant difference ($P < 0.05$).

numbers and percentages in brackets along with crude and adjusted odds ratios (COR and AOR) and their 95% confidence interval (95% CI) were noted. Factors with the *p* value of less than 0.05 were considered to have a statistically significant association with *Giardia* infection.

Results and discussion

Giardiasis is linked to the socioeconomic level of a country with prevalence ranging between 2-5% in developed countries and 20-30% in developing countries[15]. The overall prevalence of *G. lamblia* cysts in stool samples of household persons was 17.9% compared to 2.1% for *G. lamblia* trophozoite (Table 1). Nearly similar prevalence of 20% was recorded by Choy *et al.*[6]. Conversely, Johnston *et al.*[14] reported a higher rate of 40.7% among household people residing rural villages in Western Uganda using PCR, suggesting that molecular methods are more sensitive for parasites' detection than microscopy. The higher incidence rate of *G. lamblia* 24.4% in children compared to 12% for adults in this study was in concordance with Jain and Nahri[13]. This discrepancy could be related to lack of immunity, acquired resistance to infections in children, lower standards of personal hygiene and low socioeconomic status compared to adults. It was obvious from (Table 1) that *Giardia* was more prevalent in diarrhoeic versus non-diarrhoeic persons with an incidence of (28 vs 20%) in children and (15.4 vs 8.3%) in adults. These findings are consistent with an incidence of 28.1% in diarhoeic versus 19.5% in non-diarrhoeic Peruvian children reported by Cordón *et al.*[8]. Table 2 clarified that 25% of the calves' faecal samples were positive for *Giardia* cysts. Nearly similar observations were cited by Cáccio *et al.*[4]. Our results have shown a significantly higher prevalence of Giardia in diarrhoeic calves (30%) compared to (17.5%) in non-diarrhoeic ones ($P < 0.05$). These findings commensurate with Goraya *et al.*[11] who stated that buffalo calves with abnormal faeces had a significantly higher infection rate than those with normal faeces. Table 3 revealed that exposure to calves and their manure was a significant zoonotic risk factor associated with 5.5 higher odds of *Giardia* infection in persons who exposed to calves and their manure (OR=5.50; CI: 1.18- 25.71; P= 0.030) compared to persons who didn't exposed. The possible reason for these differences is that in addition to anthroponotic transmission there might be zoonotic transmission of the parasite from infected buffalo calves. Similarly, Wegayehu *et al.*[18] reported an association between *Giardia* infection and contact with cattle and their manure at household level. It is remarkable to find out that the significant association is actually in the practices of not washing hands after contact with calves and their manure (OR= 6.58; CI: 2.12-20.38; P<0.001) and not changing shoes after handling calves and their manure (OR= 5.41; CI: 1.72-17.03; P= 0.004). Consistent with our findings, Choy *et al.*[6] reported the same practices as significant risk factors for *Giardia* infection among Malaysian population. It is also worth noting that there was a significant association between

Giardia infection and the reporting of diarrhoeal symptoms in household persons exposed to calves in this study, the odds of *Giardia* infection were 4.29 times greater in persons exhibiting diarrhoeal symptoms with history of exposure to calves and their manure (OR= 4.29; CI:1.37-13.42; P=0.012) than the persons who exhibiting the symptoms without previous exposure to calves suggesting the importance of zoonotic transmission in the establishment of symptomatic giardiasis. These findings are in accordance with Coles *et al.*[7]. Interestingly, the variables that were significantly associated with *Giardia* infection in univariate analysis weren't retained by multivariate logistic regression model except not washing hands after contact with calves and their manure (AOR= 40.67; CI:3.89-424.85; P=0.002) (Table 4) due to the reduced number of individuals for which an association was found, thus hindering the disclosure of significant associations.

Recently, molecular tools have been used in assessment of the zoonotic transmission of giardiasis between animals and humans in the same focus of endemicity[10]. As shown in (Fig. 1), 292-bp fragment of SSUrRNA gene of *G. lamblia* was amplified in both isolates from household persons and calves confirming the zoonotic potential of such parasite and substantiate the role of buffalo calves in transmitting *G. lamblia* to persons living in the same households.

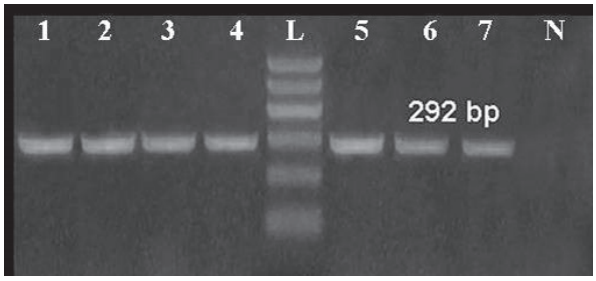

Fig. 1. Representative gel showing amplification of 292 bp of SSUrRNA gene in G. lamblia isolates;Lanes 1, 2, 3, 4 (humans' isolates); Lanes 5, 6, 7 (calves' isolates); Lane N (negative control) and Lane L (100 bp DNA ladder).

Table 3. Univariate analysis of risk factors associated with *G. lamblia* infection in 95 household persons

Variables	Exposure	No. examined (95)	No. infected (%)	Crude Odds ratio (95% CI) [a]	*P* value
1-Gender (sex)	Male	47	11(23.4)	2.14 (0.72 - 6.36)	0.17
	Female	48	6(12.5)	1.0	
2-Age group/ year	≤ 15 y	45	11(24.4)	2.37 (0.80 -7.06)	0.12
	18 -60 y	50	6(12.0)	1.0	
3-Household exposure to calves and their manure	Yes.	60	15(25)	5.50 (1.18 -25.71)	0.030*
	No.	35	2(5)	1.0	
4-Use of gloves during handling o f calves and their manure	No.	85	16(18.8)	2.09 (0.25 -17.67)	0.50
	Yes.	10	1(10.0%)	1.0	
5-Washing hands after contact with calves and their manure.	No.	28	11(39.3%)	6.58 (2.12 -20.38)	< 0.001*
	Yes.	67	6 (9.0%)	1.0	
6-Washing hands before drinking and eating	No.	23	7(30.4%)	2.71 (0.89 -8.24)	0.08
	Yes.	72	10(13.9%)	1.0	
7-Changing clothes after handling calves and their manure	No.	75	14(18.7%)	1.30 (0.33 -5.06)	0.70
	Yes.	20	3 (15.0%)	1.0	
8-Changing shoes after handling calves and the ir manure	No.	19	8(42.1%)	5.41 (1.72 -17.03)	0.004*
	Yes.	76	9(11.8%)	1.0	
9-Households exhibiting diarrhoeal symptoms with history of exposure to calves and their manure	Yes.	40	12(30.0%)	4.29 (1.37 -13.42)	0.012*
	No.	55	5(9.1%)	1.0	

*Represent statistically significant difference ($P < 0.05$).

[a] 95% confidence intervals around odds ratio (OR)

Table 4. Multivariate analysis of risk factors associated with *G. lamblia* infection in 95 household persons

Variables	AOR b	95% CI	P value
Not washing hands after contact with calves and their manure	40.67	3.89 -424.85	0.002*
Not washing hands before drinking and eating	0.11	0.01 - 1.16	0.0666

*Represent statistically significant difference ($P < 0.05$).

[b] Adjusted odds ratio (AOR).

CI Confidence interval.

Conclusions

Based on the results of this study we concluded that household livestock especially buffalo calves are important reservoir for zoonotic *G. lamblia* that can be transmitted to persons living in the same households. Contact with calves and their manure, poor personal hygienic practices including not changing shoes after handling calves and their manure are significant risk factors associated with giardiasis among persons handling calves and their manure. Moreover, not washing hands after contact with calves and their manure was confirmed as a potential significant factor strongly associated with enhancing the risk of giardiasis. Educational measures in the practice of personal hygiene and animal contact are important to reduce disease transmission and the risk of infection.

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