



Prevalence of some enteric protozoa in humans in Behera Governorate

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ABSTRACT

A total of 300 human stool specimens were collected and an interview using a pre-designed questionnaire was carried out to collect the following data including; sex, age, health status, residence and history of animal contact. Samples were subjected to routine parasitological examination for detection of diagnostic stages of *Cryptosporidium parvum*, *Giardia duodenalis* and *Entamoeba histolytica* using modified Ziehl-Neelsen staining technique, direct microscopy examination of stool suspension in physiological salt solution and microscopic examination of a direct saline (wet) mount technique. It was recorded that the incidence of *C. parvum*, *G. duodenalis* and *E. histolytica* was 15.3, 12 and 7%, respectively. Sex based incidence revealed that the incidence of previous enteric protozoa was higher in males than in females with statistical non-significant association between these incidences. Also, age based incidence revealed that the highest incidence of *C. parvum* was observed in the age group (20y - < 40y) (28.4%) followed by the age group (< 20 years) (14.6%) while the highest incidence of *G. duodenalis* was observed in the age group (> 60 y) (22.7%) followed by the age group (< 20 years) (13.5%) and finally, the highest incidence of *E. histolytica* was observed in the age group (> 60 y) (20.5%) followed by the age group (40y - > 60y) (7%) with statistical significant association between these incidences. In addition, the effects of health status, residence and the type of animal contact were investigated.

Keywords: Prevalence, Enteric, Protozoa, Humans

1. Introduction

Human diseases caused by animal parasites are still extra ordinarily common in many places, and still have great importance, especially in warm countries. This fact makes us to give the parasitic diseases special attention, since the causative agents are often not noticed by the carrier, but these diseases often lead to premature decline of vital powers or to prolonged ill health. Public health veterinarians of course bear the direct and general responsibility for knowledge of animal parasitism transmissible to man. *Cryptosporidium*, *Giardia* and *Entamoeba* species are of great public health concern as they may cause infection and severe illness in human specially children. Infections are self-limiting in people with normal immune systems but infection can be life threatening in people who have comprised immune system especially infection with *Cryptosporidium parvum* which always strongly associated with acute or chronic diarrhea in infants and HIV-infected adult patients worldwide as several recent waterborne outbreaks have shown, poses a significant threat to public health (Gabriela et al, 2005). *Cryptosporidium parvum* is one of the most important biological contaminants in drinking water that produces life threatening infection in people with compromised immune systems. Dairy calves are thought to be the primary source of *C. parvum* contamination in watersheds. Understanding the spatial and temporal variation in the risk of *C. parvum* infection in dairy cattle is essential for designing cost-effective watershed management strategies to protect drinking water sources (Szonyi et al., 2010).

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Human giardiasis caused by the intestinal flagellated *G. duodenalis* is considered as a zoonotic disease. Molecular characterization of cysts of human and animal origin represents an objective mean to validate this hypothesis, therefore calves, sheep, dogs and cats should be considered as a potential source of human infection (Van-Keulen et al., 2002). The life cycle of *Giardia* species is simple and it is included of two active trophozoite and cystic forms. This parasite transmits via fecal-oral route through direct or indirect ingestion of infectious cysts. The incubation period varies from 9 to 15 days after ingestion of cysts. Symptoms of infection are varied from the absence of symptoms to acute watery diarrhea, nausea, epigastric pain and weight loss (Giangaspero et al., 2005). Giardiasis has a global distribution and it is common in both children and adults. The prevalence of *Giardia* infection is higher in developing countries.

Domestic animals living in intimate contact with man in rural areas may have a great chance to ingest cysts of *Entamoeba histolytica*. The possibility of occasional human infection from infected animals cannot be discarded (WHO, 1979). *E. histolytica* is essentially a human parasite that can be transmitted to lower animals. It was recorded in monkeys and less frequently in dogs, cats and rats.

Cysts of *E. histolytica* are eliminated with the faeces of the host to the environment Determine the prevalence of some enteric protozoa in human inhabiting El-Behira governorate. age-associated trends of investigated protozoa in both animals and humans. Study the effects of locality, health status and sex on prevalence of investigated protozoa in humans.

2. Materials and Methods

2.1. Samples:

A grand total of 300 stool specimens were collected during the same period of time and localities of animal samples. Only one specimen was obtained from each individual and collected in sterile, dry, disposable leak proof, wide mouthed plastic containers then kept in ice box. An interview using a pre-designed questionnaire was carried out to collect the following data including age, sex, health status, residence and history of animal contact. Samples are transferred directly as soon as possible to the laboratory of Animal Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Damanhur University and were kept at 4 °C till examination.

2.2. Microscopic examination:

2.2.1. Direct smear method was performed according to (Baroody, 1946).

2.2.2. Modified formol-ether concentration (F-E) was performed according to (Ritchie, 1948).

2.2.3. Modified Ziehl-Neelsen stain was performed according to (Henrikson and Pohlenz, 1981)

Fresh stool were suspended in 2.5% potassium dichromate and homogenized then, 1 ml was used to prepare a stool smear to be stained by the modified Ziehl-Neelsen staining method. The stained fecal smear was examined microscopically under oil immersion (X100).

2.3. Molecular detection of *C. parvum* oocyst in stool by PCR:

2.3.1. Oligonucleotide primers

Cryptosporidium specific primers were designed according to Laxer et al., (1992)

Primer 1; 5' CCGAGT TTGATCCAAAAAGTTACGAA.

Primer 2; 5' TAG CTCCTCATATGCCTTATTGAGTA.

2.3.2. DNA extraction from stool specimens was performed according to Leng et al., (1996).

2.3.3. PCR amplification:

10 µl of DNA was added to microcentrifuge tube containing the PCR mixture of (PCR buffer, 2.5mM MgCl₂, 200 mM each the four deoxynucleoside triphosphates d.ATP, d.CTP, dGTP and dTTP (Amersham Biosciences), 2.5 U of Taq DNA polymerase (Amersham Biosciences), 1 mM each Cryptosporidium specific primers were added to make the final volume 100 µl. the primers were synthesized using DNA synthesizer (Geno-Mechanix). The mixture was amplified in DNA thermal cycler (Biometra). The PCR amplification was completed in 35 cycles of 2.5 minutes at 94C (denaturation), 2 minutes at 59 C (annealing) and 2.5 minutes at 72C (extension) followed by a final incubation at 72 C for 10 minutes. Finally, 30 µl from each PCR amplification were electrophoresed on polyacrylamide gel, stained with ethidium bromide solution (0.5 µg/ml), visualized under an ultraviolet transilluminator and photographed. Visible bands of appropriate size of 452 bp (*C. parvum*) were considered positive.

2.4. Statistical analysis:

It was made using Chi² test to examine the significance differences of the detection rate of antibodies among different groups studied according to SAS, (2014).

3. Results and Discussion

The burden of zoonotic disease falls disproportionately on poor people in rural areas who live in close contact with their animals (Maudlin et al., 2009). Livestock serve many functions for people in rural areas including: food source, traction/transport, manure, dowry, and financial security (Muma et al., 2014). Zoonotic diseases impact both human health and also livelihoods as disease in animals can result in reduced livestock productivity (Maudlin et al., 2009).

The recorded results in Table (1) revealed that the prevalence of *C. parvum* depending on the results of Modified Ziehl-Neelsen staining technique was 15.3%, in the examined stool specimens of human while the prevalence of *G. duodenalis* was 12% depending on the results of direct microscopy examination of stool suspension in physiological salt solution and finally, the prevalence of *E. histolytica* was 7% depending on the results of microscopic examination of a direct wet mount technique. Concerning the prevalence of *C. parvum*, it was higher than that recorded by Samaha et al., (2012) (7%) and Helmy et al., (2013) (6.7%).

Sex based prevalence of enteric protozoa in human revealed that the prevalence of *C. parvum* was higher in males (15.8%) than in females (15%) with statistical non-significant association between them. Also, the prevalence of *G. duodenalis* was higher in males (12.5%) than in females (11.7%) with statistical non-significant association between them and lastly, the prevalence of *E. histolytica* was higher in males (10.8%) than in females (4.4%) with statistical non-significant association between them. This finding agreed with Samaha et al., (2012) who found that the prevalence of *C. parvum* was higher in males (12.5 %) than in females (10.9 %) while it disagreed with Zidan (2006) who recorded an prevalence of 10.6 % in male children and 13.34 % in females and his statistical analysis showed that there was no association between sex of children and infection with *C. parvum*.

Das et al., (1993) and Shehata, (1997) indicated that no sex specific predilection for Cryptosporidium was observed in either diarrheic or non-diarrheic groups of investigated children.

The prevalence of *G. duodenalis* in children was 11.3 % which was nearly similar to that obtained by Omar et al. (1991) (10.9%) and Zidan (2006) (10.8 %), while it was lower than that obtained by Meloni et al., (1993) (32.1%), Curtale et al. (1998) (24.7%) and Bhandari et al. (1999) (20 %). WHO, (1979) stated that domestic animals living in close contact with man in rural areas may have a great opportunity to ingest cysts of *E. histolytica* and the possibility of occasional human infection from infected animals cannot be discarded.

The prevalence of *E. histolytica* infection in stool samples from children was 7 % which was higher than that obtained by Rayan et al., (2010) (4.2 %), while it was lower than that obtained by Surtiasuti (2006) (14.53 %), Menghi et al., (2007) (24.1%), Ben Ayed et al., (2008) (28 %) and Ouattara et al., (2008) (11.3 %).

As shown in Table (2), age based prevalence of enteric protozoa in human revealed that the highest prevalence of *C. parvum* was observed in the age group (20y - < 40y) (28.4%) followed by the age group (< 20 years) (14.6%) then the age group (> 60 y) (9%) and finally the age group (40y - > 60y) (7%) with statistical significant association between these prevalences while the highest prevalence of *G. duodenalis* was observed

in the age group (> 60 y) (22.7%) followed by the age group (< 20 years) (13.5%) then the age group (40y - > 60y) (10.5%) and finally the age group (20y - < 40y) (6.2%) with statistical significant association between these prevalences and finally, the highest prevalence of *E. histolytica* was observed in the age group (> 60 y) (20.5%) followed by the age group (40y - > 60y) (7%) then the age group (20y - < 40y) (4.9%) and finally the age group (< 20 years) (2.2%) with statistical significant association between these prevalences.

The detection rate in age group less than 15 years old was lower than recorded by Helmy et al. (2013) (49.1 %), Mittal et al. (2014) (36%), Ahmed et al. (2016) (17.2%) and Ghoneim et al. (2017) (33.9%).

This result agreed with Zidan (2006) who found that the highest prevalence was in the age group (<2 years old) (20.2 %) followed by the age group (3- 4 years) (7.5 %) then the age group (< 6 years old) (5.5 %) and Samaha et al., (2012) who recorded that the highest prevalence was observed in the age group (<2 years) (18.2 %) followed by the age group (2- <4 years) (9.6 %) and finally the age group (4- <6 years) (5.6 %). These results indicated that the age of children at risk is an important factor in *C. parvum* infection. These results were confirmed by the finding of Das et al. (1993) who observed that the highest detection rate of *C. oocysts* was in the first two years of life in both diarrheic and control children and Henry et al. (1995) who concluded that the highest risk of persistent diarrhea was in children of 2-3 years.

The effect of health status on the prevalence of enteric protozoa in human was tabulated in Table (3). It clarified that diarrheic individuals showed higher prevalence of *C. parvum* (15.5%) compared to apparently healthy animals (5.9%) with statistical non-significant association between these prevalences. Also, diarrheic individuals showed higher prevalence of *G. duodenalis* (11.6%) and *E. histolytica* (13.6%) compared to apparently healthy individuals with statistical non-significant association between these prevalences.

As presented in Table (4), the effect of residence of investigated individuals on the prevalence of enteric protozoa in human clarified that higher prevalence of *C. parvum* (15.9%) occurred in those who lived in rural areas compared to those who lived in urban area (8.9%) with statistical non-significant association between these prevalences. On contrary, higher prevalence of *G. duodenalis* (23.2%) and *E. histolytica* (21.4%) was observed in those who lived in urban areas with statistical non-significant association between these prevalences.

As shown in Table (5), the effect of type of animal contact of investigated individuals on the prevalence of enteric protozoa in human clarified that higher prevalence of *C. parvum*, *G. duodenalis* and *E. histolytica* occurred in those who informed that there was no animal contact with statistical non-significant association between these prevalences.

These results confirmed by Hira et al., (1989) who stated that contaminated water supplies and contact of animals such as sheep and goats were of the known risk factors facilitating transmission. Moreover, Keusch et al. (1992) indicated that persons at greatest risk were immunocompromised adults and children, especially those with AIDS, children in day care and travelers to endemic regions.

It was clear that animal contact did not affect prevalence of *C. parvum* where prevalence was lower in persons with history of animal contact (14.7 %) than others with no history of animal contact (17.1 %). This finding disagreed Zidan (2006) who recorded a prevalence of 16.7 % in contact group and 4.2 % in non-contact group. On contrary, these findings agreed with Shehata (1997) who reported that there was no significant relationship between the infection with *C. parvum* and the history of animal contact and believed that other means of transmission were more important to cause infection with *Cryptosporidium* spp. than zoonotic transmission.

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Table (1): Prevalence of enteric protozoa in human stool specimens in relation to sex

Sex	No. of examined samples	Enteric protozoa					
		<i>C. parvum</i>		<i>G. duodenalis</i>		<i>E. histolytica</i>	
		Positive	%	Positive	%	Positive	%
Female	180	27	15.0	21	11.7	8	4.4
Males	120	19	15.8	15	12.5	13	10.8
Total	300	46	15.3	36	12.0	21	7.0
Chi ² value		0.15 NS		0.16 NS		1.62 NS	

NS = Non-significant at (P < 0.05).

Table (2): Prevalence of enteric protozoa in human stool specimens in relation to age

Age group	No. of examined samples	Enteric protozoa					
		<i>C. parvum</i>		<i>G. duodenalis</i>		<i>E. histolytica</i>	
		Positive	%	Positive	%	Positive	%
< 20 y	89	13	14.6	12	13.5	2	2.2
20y - < 40y	81	23	28.4	5	6.2	4	4.9
40y - > 60y	86	6	7.0	9	10.5	6	7.0
> 60y	44	4	9.0	10	22.7	9	20.5
Total	300	46	15.3	36	12.0	21	7.0
Chi ² value		6.13**		7.29**		4.33**	

NS = Non-significant at (P < 0.05).

Table (3): Prevalence of enteric protozoa in human stools in relation to health status

Health status	No. of examined samples	Enteric protozoa					
		<i>C. parvum</i>		<i>G. duodenalis</i>		<i>E. histolytica</i>	
		+ve	%	+ve	%	+ve	%
Diarrheic	132	36	15.5	27	11.6	18	13.6
Apparently healthy	168	10	5.9	9	5.4	3	1.8
Total	300	46	15.3	36	12.0	21	7.0
Chi ² value		X ² =3.168 0.075 NS		X ² =2.004 0.367 NS		X ² =0.540 0.463 NS	

NS= Non-significant at (P >0.05)

Table (4): Prevalence of enteric protozoa in human stool specimens in relation to residence

Residence	No. of examined samples	Enteric protozoa					
		<i>C. parvum</i>		<i>G. duodenalis</i>		<i>E. histolytica</i>	
		Positive	%	Positive	%	Positive	%
Rural	244	39	15.9	23	9.4	9	3.7
Urban	56	5	8.9	13	23.2	12	21.4
Total	300	46	15.3	36	12.0	21	7.0
Chi ² value		X ² =0.829 0.843 NS		X ² =1.001 0.317 NS		X ² =1.859 0.868 NS	

NS= Non-significant at (P >0.05)

Table (5): Prevalence of enteric protozoa in human stools of in relation to animal contact

Animal contact	No. of examined samples	Enteric protozoa					
		<i>C. parvum</i>		<i>G. duodenalis</i>		<i>E. histolytica</i>	
		Positive	%	Positive	%	Positive	%
Contact	224	33	14.7	25	11.2	10	4.5
Non-contact	76	13	17.1	11	14.5	11	14.5
Total	300	46	15.3	36	12.0	21	7.0
Chi ² value		0.02 NS		1.31 NS		1.54 NS	

NS= Non-significant at (P >0.05)



Photo (2): Entamoeba histolytica cyst in fecal samples



(A)

Photo (3): Giardia cyst (A) and Giardia trophozoite (B) in fecal samples



(B)

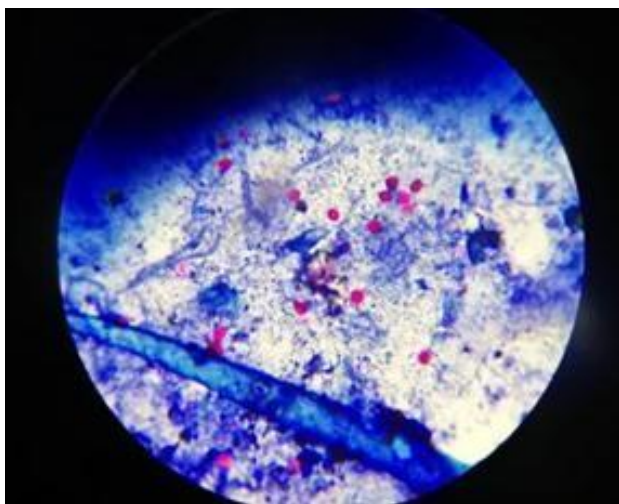


Photo (1): *C. parvum* oocyst in fecal samples stained by MZS