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An epidemiological study on giardiasis in cattle and humans at Beni-Suef Governorate

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The present study was conducted to assess the prevalence of *Giardia* species infection in cattle and human. One hundred of animal fecal samples and 139 human stool samples were collected from different veterinary clinics and its related hospitals respectively. All samples were undergone to microscopically examination by; direct smears in 0.90% Na Cl solution, Lugol's iodine stain for cyst detection and formol-ether concentration. 9 (28.1%) calves from 32 were positive in microscopic examination by the used techniques. 25% of the examined fecal samples of cattle (17/68) were containing cysts of *Giardia* species by microscope. 39 of 139 (28.1%) of human stool samples were found infected by this protozoon. Regarding the sex of human cases, 26.30% of examined males were positive while 30.20% of females were positive. The age factor in human infection was clear; the age group of 11 to 20 years were the more infected than the other group (1-10ys). There is no relation between form of human stool and infection rate. ELISA kits confirmed that 6 % of animal cases and 15.8% of human were positive. The epidemiological aspects were discussed in the study.

Giardia duodenalis is a common intestinal protozoan parasite that infects a wide variety of domestic and wild mammals as well as humans (O'Handley and Olson 2006). Transmission of the parasite is dependent upon ingestion of cysts, which are excreted in the feces of infected hosts. While the direct fecal-oral route of transmission is important, waterborne transmission is a major route for human infections, specifically from *G. duodenalis* contaminated surface water (Dixon, 2003, van Keulen*et al.*, 2002). Typically, *G. duodenalis* infects the small intestine of the host. Clinical giardiasis in humans is recognized by diarrhea (acute or chronic), dehydration, abdominal pain, nausea, vomiting, and weight loss (Thompson and Monis 2004). Giardiasis in cattle is usually subclinical in adult cows. Calves may experience diarrhea; however, subclinical infections are common, probably depending on host, parasite, and environment interactions. Mixed infections with other protozoan and viral pathogens are common and may be responsible for clinical signs encountered (O'Handley*et al.*, 1999).

Six species of *Giardia* are recognized on the basis of morphological characteristics and host occurrence (Thompson, 2003). The lack of morphological differences between the genetic variants found in mammals has resulted in an informal categorization of these genotypes based on genetic differences. Cattle are susceptible to infection with two genotypes of *G. duodenalis*: the zoonotic genotype Assemblage A, or the livestock genotype, Assemblage E (Thompson, 2003; Olson, *et al.*, 2004). Cattle may be a potential source of human *Giardia* infection through direct contact, or more importantly through contamination of surface water supplies Weniger *et al.*, (1983), Craun (1986), Gradus (1989), Craun (1990), Le chevalier *et al.*, (1991); even few calves infected with genotypes in assemblage A could pose a significant public health risk and may but producers, and other members of the community at risk (Santin *et al.*, 2012).

So this study was conducted in Beni-Suef Governorate to determine the prevalence and zoonotic potential of giardiasis in domestic cattle and humans.

Material and methods

Animals' fecal samples. A total of 100 fecal samples were collected from; different veterinary clinics in the nearest villages to Beni-Suef town. These samples are 68 dairy cattle and 32 calves. Every fecal sample was collected per rectum using disposable latex glove then the feces are put into individual plastic containers. The fecal samples were transferred to the laboratory in ice bags at 4ºC. Each sample was identified by animal number, age, sex and all data of him.

Human stool samples. The human stool samples were collected from the hospitals that were the only way for the inhabitants of these villages near to Beni-Suef town for medical care.

A total of 139 human stool samples of patients suffering from gastrointestinal disturbance and visiting the outpatient's clinic laboratory for examination were collected. The data of the patients were written for each samples and its residence (age, sex, form of the stool, etc...). Samples were collected in accordance with WHO guidelines on the collection of faecal samples WHO (1991). From the samples; 112 were normal formed or semi formed stool and 27 were diarrheic stool. Each sample was labeled then was sent to laboratory for further examination.

Microscopical technique for examination. Three techniques by 3 steps for examination of each sample were done. Direct microscopic smear in saline (0.90% w/v NaCl solution) was assumed then was examined microscopically. Then; Lugol's iodine was performed for the detection of parasites (trophozoites, cysts) and lastly formol-ether concentration method was employed according to Ridley and Hawgood (1956).

Immunologic detection of Giardia species antigen in feces. It was performed using *Giardia* specific coproantigen ELISA (Immunospec Corporation) to detect different soluble antigens dispersed in fecal matter rather than detecting cysts, trophozoites.

The procedure was conducted according the manufactured. This briefly was; the fecal samples were prepared by dilution buffer. All wells were filled by 50 ul dilution buffer. Then 50 ul of each sample was put in each well. Incubation for 60 min at room temperature was done then washing occurred. Add 2 drops of enzyme conjugate to each well. Then it was incubated for 30 minutes at room temperature, and then washed. Two drops of chromogen were added and incubation for 10 min at room temperature. Then, 2 drops of stop solution was put. Mixing was done and read the reaction within 5 minutes after adding stop solution. The results were read at a dual wavelength of 450nm. The readings were compared to the negative control and positive control.

Results

The microscopic examination of fecal samples from calves showed higher percentage of positivity (28.1%) than cattle (25%) as in (Table 1). Concerning the humans stool samples examination reveals positive rate of 28.1% with higher positivity rate in the age group of 11-20 years irrespective to presence or absence of diarrhea with slight rise in female positivity than male (Table 2). The ELISA confirmed 6% of animal cases and 15.8% of human cases as shown in (Table 1 and 3).

Table (1): Results of Microscopical and ELISA examination of animal fecal samples.

Animal	Test used			
	Direct smear &formol-ether concentration technique		ELISA	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Cattle	17 (25%)	51 (75%)	3(4.4%)	65 (95.6%)
Calves	9 (28.1%)	23 (71.9%)	3(9.4%)	29(90.6%)
Total	26 (26%)	74(74%)	6(6%)	94(94%)

Table (2): Distribution of infection in relation to age and sex using microscopic examination of direct smear &formol-ether concentration technique.

Age groups	No. of samples		Positive samples by direct smear &formol-ether concentration technique				Total positive (%)
			Male		Female		
	Male	Female	Diarrheic stool	Normal stool	Diarrheic stool	Normal stool	
1-10	45	43	2	8	1	10	21(23.8%)
11-20	31	20	2	8	1	7	18(35.3%)
Subtotal	76	63	4	16	2	17	
Total (%)	139(100%)		20(26.3%)		19(30.2%)		39(28.1%)

Table (3): Comparison of coproantigen ELISA results with microscopical examination.

Test	Positive (%)	Negative (%)
Direct smear and formol-ether concentration technique	39(28.1%)	100(71.9%)
ELISA	22(15.8%)	117(84.2%)

Discussion

The prevalence of *G. duodenalis* infection is high in cattle throughout the world and all age-groups can be infected (Langkjaer *et al.,* 2007). The present results show infection rate of 26% with higher percentage of positivity in calves (28.1%) than cattle (25%). This result showed that the domestic animals such as cattle especially calves may serve as reservoir hosts for *Giardia* infection provide the risk of the infection with subsistence farming and animal husbandry being the major occupation of the people which in accordance to Buret *et al.,* (1990) who found that the prevalence of giardiasis was 10.4% in cattle and 27.7 % in calves in Canada and postulated that domestic ruminants may be a reservoir for human infection and vice versa, thus classifying giardiasis as a zooanthroponotic disease. Also, Ilburg *et al.,*(1996) found Giardia cysts in 7.6% of 92 samples from 59 cows and 33 calves in Denmark. Six species of *Giardia* are recognized on the basis of morphological characteristics and host occurrence (Thompson, 2003). The lack of morphological differences between the genetic variants found in mammals has resulted in an informal categorization of these genotypes based on genetic differences. Cattle are susceptible to infection with two genotypes of *G. duodenalis*: the zoonotic genotype Assemblage A, or the livestock genotype, Assemblage E (Thompson, 2003; Olson, *et al.,* 2004). *Giardia* has the potential to cause clinical disease in cattle and to be transmitted to other animal species and humans; in addition Olson *et al.,* (1997) stated that *Giardia* infections are highly prevalent in dairy calves (73%) in Columbia and should be considered in animals with diarrhea or failure to thrive. Recent attention has also focused on the widespread and unexpectedly high levels of infection of *Giardia* in young livestock, particularly calves O'Handley *et al.,* (2000a). A number of North American studies have demonstrated a high prevalence of *Giardia* in dairy calves, with infection rates of 100% in some herds Xiao and Herd (1994) and Handley *et al.,* (1999) . As *Giardia* isolates recovered from ruminants are morphologically and antigenically identical to isolates recovered from humans Buret *et al.,* (1990). Calves in dairy herds may harbor two genotypes, one of which is capable of causing infection in humans. Although the livestock genotype of *Giardia* appears to be the most common encountered one in cattle, studies in Canada and Australia have shown that a small proportion of cattle in a herd, <20%, may harbor genotypes in assemblage A, the most common zoonotic genotype affecting humans **O'**Handley *et al.,* (2000b) and Trout *et al.,* (2005), also Santin *et al.,*(2012) stated that even few calves infected with genotypes in assemblage A could pose a significant public health risk and may but producers, and other members of the community at risk while other studies in the United States, Canada, and the United Kingdom did not show such an association Xiao and Fayer. (2008).

Cysts are discharged in the feces of infected cattle and are of primary importance for the dispersal and survival of the parasites. Transmission can be direct from host to host, by ingestion of fecal contaminated food or water, or, as with other fecal transmitted parasites mechanical insect vectors are likely to play a role in transmission. Limiting factors for oocyst and cyst survival are high temperatures and desiccation. Transmission is likely to be direct between infected animals since environmental contamination on farms with oocysts and cysts would be insufficient to account for the high levels of infection seen in cattle, particularly with *Giardia*. Although the transmission process is complex and the risk is low, there is a definite potential for *Giardia* and *Cryptosporidium* contamination of ground and surface waters from livestock operations. Management of fecal waste is crucial when water runoff can reach receiving surface water or contaminate groundwater. There are major concerns with applying fresh animal manure to fertilize agricultural land due to the potential for fecal pathogens to reach surface and/or groundwater. It is believed that the primary modes by which parasites such as *Giardia* and *Cryptosporidium* are transported to surface water are via the drainage from manure storage areas, direct contact by cows with water,

runoff from fields on which manure has been spread and wash from manure-laden soil. Parasites such as *Giardia* and *Cryptosporidium* have been associated with contamination of fruits and vegetables through contaminated irrigation water and manure fertilizer. *Giardia* cysts have been shown to be viable for up to 84 days in cold river and lake water but are eliminated within a week when frozen or desiccated (O'Handley*et al.*,2000a; Olson *et al.*,1999).

Concerning the stool sample examination the results revealed positive rate of (28.1%) with higher positivity rate in the age group of 11-20 years (35.3%) irrespective to presence or absence of diarrhea with slight rise in female positivity(30.2%) than male (26.3%) which revealed that *Giardia* infection may either be present sub-clinically or the parasite have partial pathogenicity or the majority of the patients within the study area are asymptomatic carriers of a non-pathogenic strain, this in agreement with Haque *et al.,* (2005) who stated that different genotypes of *Giardia lamblia* (Assemblage A and Assemblage B) has been reported in Bangladesh with the Assemblage A genotype more associated with diarrhoea than the Assemblage B genotype. The incidence of infection increased significantly with age. The majority of the patients in this study were children of school and pre-school age and thus they have very active playing habits in and out of school. These children normally play in the soil which harbors this parasite cyst and are less mindful of some very important personal hygiene practices such as washing of hands with soap and water before eating, after playing in the soil and after visiting the toilets, also, they may buy a lot of food from streets vendors some of whom do not practice proper personal hygiene and may be carriers of some infective parasites the same was explained by Ayeh *et al.,* (2009) , Nyarango *et al.,* (2009), also Mahmud *et al.,*(1995) who suggested that in addition to age of infants, poverty, low education, gender discrimination, and certain environmental conditions potentiated the risk for developing the 1%infection in addition Addy *et al.,* (2004) and Wongjindanon *et al.,* (2005) explained that the nature of everyday activities bring people, especially children, into close contact with natural sources of soil and water, therefore increasing their risk of ingestion of infective stage parasites. This study shows contrary to previous suggestions that giardiasis was highest only among children of pre-school age who are usually in child care settings Heidari and Rokni (2003). Higher isolation rates were reported in Egypt (44%) Zaki *et al.,* (1986), children in the aborigine community in Pahang, Malaysia (44.1%) Noor *et al.,* (2007), and children in Amman, Jordan (78%) Shakkoury and Wandy (2005). Even lower prevalence have been reported in other areas such as diarrheal children in Kumasi, Ghana (11.0%) Addy and Aikins (1986) and pre-school children in Gaza, Palestine (10.3%), Al-Hindi and El-Kichaoi (2008). Both studies showed no clear trend in prevalence with age. In agreement with our results Wongjindanon *et al.,* (2005) found that *Giardia infection* was observed almost three times more in asymptomatic children (9.7%) than in symptomatic children (3.7%)

The study also revealed that ELISA is an easy, rapid, sensitive and specific procedure for confirming the diagnosis of suspected cases of giardiasis. It should be a valuable diagnostic aid under field conditions as well as in the laboratory as deduced by Vinayak *et al.,* (1991) who suggested that ELISA appears to be a simple, rapid, and accurate method for the detection of *G. lamblia* in unprocessed stool samples, also Danciger, (1975) and Faust (1970) who found that diagnosis of *Giardia* infection by microscopic examination for ova & parasite (O&P) is a laborious process. Moreover Burke (1975) ,Healy (1979) and Kamath and Murugasu (1974) found that iodine stained wet smears, gimsa stained cyst concentrates prepared by formol-ether concentration are standard methods of stool preparation used to increase the sensitivity of *Giardia* detection but even after application of these techniques ,the sensitivity of microscopic examination is dependent upon the skill of the microscopist, on the other hand Jelinek *et al.,* (1996) who stated that the coproantigen-ELISA is especially advantageous in situations where only a single stool sample can be examined. It was worthy to mention that ELISA result was lower than microscopic examination; this may attributed to; chronic giardiasis has local reaction in the intestine, also the detection of this kit was directed to IGg not any other `immnuoglobulines.

Finally, cattle especially calves may a role in the dissemination and epidemiology of human giardiasis.

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