

Role of animal in occurrence of some zoonotic enteric protozoa in different areas of Nile Delta

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A total of 807 stool and fecal samples (251 stool samples from diarrheic children under six years old, 254, and 250 fecal samples from diarrheic and apparently healthy pre-weaned calves and lambs, respectively in addition to 50 fecal samples from dogs) were collected from different localities in Behera and Menoufia Governorates for detection of *Cryptosporidium spp.*, *Giardia spp.* and *Entamoeba histolytica*. *Cryptosporidium spp.* has been detected by using modified Ziel-Nelssen Stain (MZN) in 30 (11.95%); 26 (10.24%); 31(12.4%) and 2(3.84%) of the examined stool and fecal samples from children, calves, lambs and dogs, respectively in both Governorates. There were significant relationships between infection of the examined calves with *Cryptosporidium parvum* and their age and healthy status. The same relation was noticed in concern with the examined children. Results of MZN were confirmed by using ELISA which was found sensitive (overall sensitivity 96.6%). In spite of the higher sensitivity of PCR than MZN for detection of *C. parvum* in fecal specimens especially when oocysts are scanty, the high cost of reagents and lack of expensive instruments which are not available in all clinical laboratories render MZN staining technique acceptable and reliable. By using direct smear and formal ether method, *Giardia intestinalis* has been detected in {27(10.75%); 51(20.08%); 63(29.2%) and 5 (9.61%)} of stool and fecal samples from the examined children, calves, lambs and dogs, respectively from both Governorates. Calves, lambs and dogs seem to be important sources for *Giardia intestinalis* to man. *Entamoeba histolytica* has been detected in {19(7.56%); 0 (0),0(0) and 2(3.84%) of stool and fecal samples of the examined children, calves, lambs and dogs, respectively in both governorates. Dogs are regarded as an important source of *Entamoeba histolytica* to man.

Presence of *Cryptosporidium parvum*, *Giardia sp.* and *Entamoeba* species in the environment especially water and in mammals including man has received increased attention in recent years (Tavarez *et al.*, 1991 and Fayer *et al.*, 2000). Domestic animals living in intimate contact with man in rural areas in Egypt constitute a big risk for transmission of infection with these protozoal agents to man. These protozoa are of public health concern as they may cause infection and severe illness in human. Infections are self limiting in people with normal immune system but infection can be life threaten in people who have comprised immune system (Gabriela *et al.*, 2005). In Egypt, more than 50% of deaths among children lower than two years are due to diarrheal diseases (Mostafa and Zaki, 1992; Fayer, 2000). Although *Cryptosporidium* infection of livestock may have an important economic impact on farmers because of high morbidity. The excreted cryptosporidial oocyst and *Giardia* cysts with feces of infected animals, particularly calves can be a source of human infection. People in rural areas are also more

exposed to ingest cysts of *Entamoeba histolytica* from infected animals (WHO, 1979).

Materials and methods

This study was carried out in some rural areas of Behera and Menoufia Governorates throughout a period of one year. A total of 807 stool and fecal samples were collected from different localities in Behera and Menoufia Governorates (251 stool samples from diarrheic children under six years old attending pediatric hospitals, 254, and 250 fecal samples from diarrheic and apparently healthy pre-weaned calves and lambs, respectively in addition to 52 fecal samples from dogs in Behera Governorate only). All the collected samples were identified for the locality, sex, age, health status and character of the fecal matter of children and animals. In the laboratory, 1 g of each stool and fecal sample was emulsified in 10% formalin solution and preserved until performing MZN technique and detection of *Giardia spp.* and *Entamoeba histolytica* cysts, Approximately 5 g of each stool and fecal sample was mixed with 2.5% potassium dichromate solution and kept at

4°C for detection of *Cryptosporidium parvum* by ELISA.

Modified Ziel-Nelssen technique (MZN). *Cryptosporidium* spp. oocysts were stained with modified Ziel-Nelssen stain and examined microscopically according to (Henrikson and Pohlenz, 1981).

ELISA. The test was performed for detection and confirmation of the MZN positive samples using (Ridiascreen test C-1201 GmbH, Darmstat, Germany) according to (Anusz *et al.*, 1990).

PCR. It was applied for detection and identification of *Cryptosporidium parvum* in stool and feces of some children, calves, and lambs. 1 g of stool or fecal sample was diluted 1:5 with *Cryptosporidium* lysis buffer (CLB), mixed well and centrifuged at 15000 X g for 5 minutes. The supernatant was removed and the pellet was resuspended with 100 µl of CLB (Leng *et al.*, 1996). *Cryptosporidium* specific Oligonucleotide primers were selected according to (Laxer *et al.*, 1992). Primer 1. 5' CCGAGT TTGATCCAAAAAGTTACGAA. Primer 2. 3' TAG CTCCTCATATGCCTTGAGTA. PCR was amplified in DNA thermal cycler (Biometra) in 35 cycles of 2.5 minutes at 94°C (denaturation), 2 minutes at 59°C (annealing) and 2.5 minutes at 72°C (extension) followed by final incubation at 72 for 10 minutes. 30 µl from each PCR amplified amplicons were subjected to gel electrophoresis, stained with ethidium bromide and visualized under ultra violet transilluminator. Statistical analysis was computed using Chi-square test according to (Hill, 1979).

For detection of *Giardia intestinalis* and *Entamoeba histolytica*: Direct smear methods according to (Baroody, 1946) and Modified formol-ether concentration according to (Henrikson and Pohlenz, 1981) were performed.

Results and Discussion

Human enteric diseases caused by animal protozoal agents are common in many places especially the rural areas of Egypt. Role of animals harboring *C. parvum*, *Giardia lamblia* and *Entamoeba histolytica* in transmission of infection to human in different localities of Menoufia and Behera Governorates was studied.

Table 1 and Fig. 1, show the occurrence of *Cryptosporidium parvum* in stool and fecal samples of children, calves, lambs and dogs as examined by MZN and ELISA. Out of 251; 254; 250 and 52 stool and fecal samples, *C. parvum* was detected by MZN technique in 30 (11.95%); 26(10.24%); 31(11.6%) and 2(3.84%) samples, respectively. Isaacs *et al.*, (1985); Hunt *et al.*,

(2000) stated that MZN staining technique has been widely used as a reliable method for detection of *Cryptosporidium* oocysts in fecal samples since it allows observation of the protozoan oocysts at lower magnification power and solves the problem of differential diagnosis related to the presence of yeasts. Nearly similar results were recorded by (Soliman 1992; Uga *et al.*, 2000; Majewska *et al.*, 2000; Huber *et al.*, 2005). In spite of detection of *C. parvum* in higher rates by other researchers (Abo El-Magd and Haiba, 1989; Naciri *et al.*, 1999; Wadi *et al.*, 2000) stated that collection of samples from diarrheic and non diarrheic calves resulted in lowering the percentage of *C. parvum* detection. MZN technique Positive samples were re-examined by ELISA which revealed detection of the protozoan in 30(11.95%); 25(9.83%); 29(11.6%) and 2(3.84%) in children, calves, lambs and dogs samples, respectively. The sensitivity of ELISA in detection of the protozoan antigens in stool and fecal samples was compared with that of MZN technique. The sensitivity of ELISA was 100; 96; 94 and 100%, respectively. Although, McClusky *et al.*, (1995) indicated moderate agreement between two diagnostic methods, with the ELISA being the more sensitive, Majewska *et al.*, (2000) showed that both methods had the same sensitivity.

The association between occurrence of *C. parvum* infection in calves and lambs with their age was studied (Table 2, Fig. 2). The examined calves were grouped according to their age during the course of the study into three groups. *C. parvum* oocysts were detected in 20; 3 and 3 samples out of 132 (<1 month old); 59 (<2 months old) and 63 (<3 months old), respectively. In addition, *Cryptosporidial* oocysts were detected in 21 (19.63%); 7 (8.75%) and 3 (4.76%) out of 107 (<1 month old); 80 (<1 month old) and 63 (<3 months old), respectively in examined lambs. It was obvious in both animal species that the highest occurrence of *C. parvum* infection in younger age group (<1 month old). The Ch-square value (X²) was 7.234* for the calves group and 7.234 * (P<0.01) for the lambs group. Garber *et al.*, (1994) reported that clinical infections with *C. parvum* in cattle are largely confined to new born calves (7-21 days old). In addition, Villacorta *et al.*, (1991); Scott *et al.*, (1994); Noordeen *et al.*, (2000) indicated that excretion of oocysts has been found in apparently healthy cows and lambs.

The association between occurrence of

Table (1): Occurrence of *Cryptosporidium parvum* in stool and fecal samples of children, calves, lambs and dogs in Behera and Menioufia governorates.

Host	MZN		ELISA		Total	ELISA sensitivity
	+ ve cases	%	+ ve cases	%		
Children	30	11.95	30	11.95	251	100%
Calves	26	10.24	25	9.83	254	96%
Lambs	31	11.6	29	11.6	250	94%
Dogs	2	3.84	2	3.84	52	100%

Table (2): Association between occurrence of *C. parvum* in calves and lambs with their age.

Age group	Calves			Lambs		
	No.	+Ve	%	No.	+Ve	%
<1 month old	132	20	15.15	107	21	19.63
<2 months old	59	3	5.08	80	7	8.75
<3 months old	63	3	4.76	63	3	4.76
Chi-square value	X2 = 7.234 *		X2 = 9.5** (P< 0.01)			

Table (3): The association between occurrence of *C. parvum* and health status of the examined calves.

Health status	+Ve cases		-Ve cases		Total No.
	No.	%	No.	%	
Diarrheic calves	21	14	129	86	150
Apparently healthy calves	5	4.8	99	95.2	104
Chi-square value	X2=5.647*		P<0.05		

Table (4): The association between age of the examined children in Behera and Menioufia governorates and occurrence of *C. parvum* in their stool.

Age group	+Ve cases		-Ve cases		Total No.
	No.	%	No.	%	
< 2 years old	20	20.20	79	79.80	99
3-4 years old	6	7.50	74	92.50	80
< 6 years old	4	5.55	68	94.44	72
Chi-square value	X2=10.71**		P<0.01		

Table (5): The association between history of contacts with animals and infection of children with *C. parvum*.

Group	+Ve cases		-Ve cases		Total No.
	No.	%	No.	%	
Contacts with animals	26	16.66	130	83.33	156
None contacts with animals	4	4.2	91	95.80	95

Chi-square value X2 = 8.51**

Table (6): Confirmed results of MZN technique by PCR for detection of *C. parvum* in children, calves and lambs Behera and Menioufia governorates.

Host	No. +Ve samples as tested by MZN	No. +Ve samples as tested by PCR	Sensitivity
Children	4	4	100%
Calves	4	4	100%
Lambs	3	3	100%

Table (7): Occurrence of *Giardia lamalia* infection in stool and fecal samples from children, calves, lambs and dogs.

Host	+Ve cases		-Ve cases	
	No.	%	No.	%
Children	26	10.75	224	89.25
Calves	31	20.08	203	79.92
Lambs	63	29.20	187	70.8
Dogs	5	9.61	47	90.38

C. parvum and health status of the examined

Table (8): Occurrence of *Entamoeba histolytica* infection in stool and fecal samples from children, calves, lambs and dogs.

Host	+Ve cases		-Ve cases		Total No.
	No.	%	No.	%	
Children	19	7.56	232	92.44	251
Calves	0	0	254	100	254
Lambs	0	0	250	100	250
Dogs	2	3.84	50	96.16	52

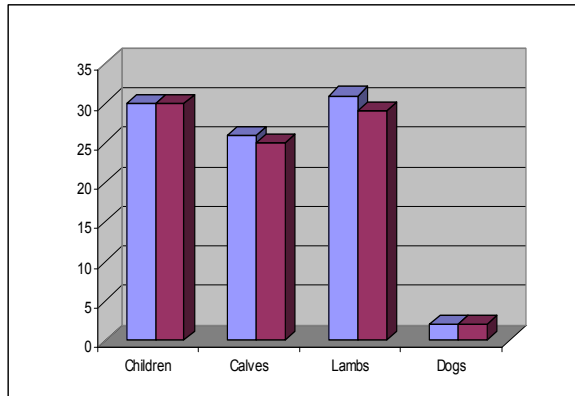


Fig. (1): Occurrence of *Cryptosporidium parvum* in stool and fecal samples of Children, calves, lambs and dogs in Behera and Menioufia governorates.

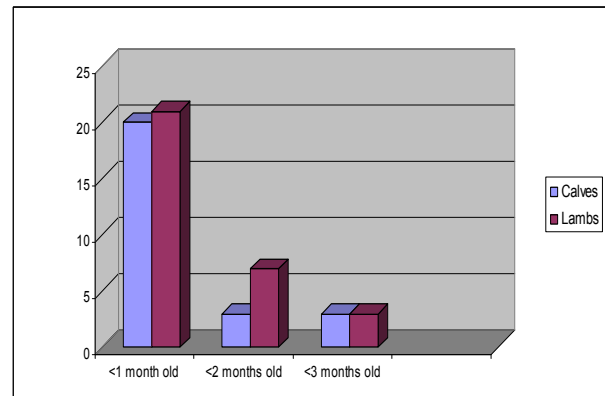


Fig. (2): Association between occurrence of *C. parvum* in calves and lambs with their age.

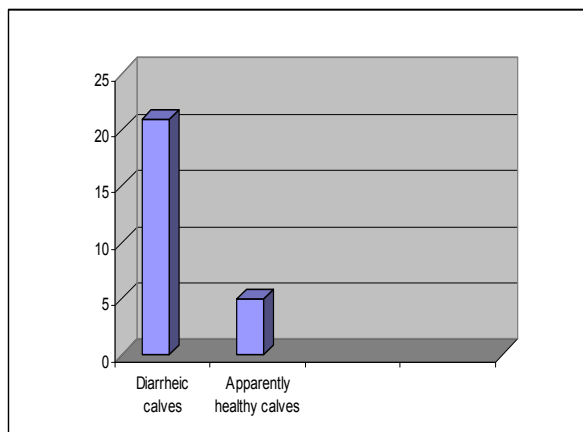


Fig. (3): The association between occurrence of *C. Parvum* and health status of the examined calves.

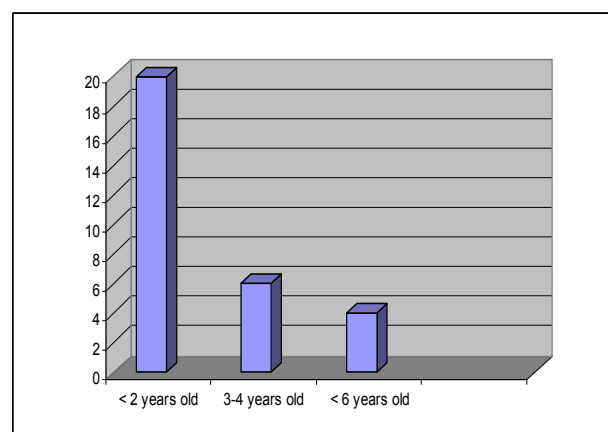


Fig. (4): The association between age of the examined children in Behera and Menioufia governorates and occurrence of *C. parvum* in their stool.

calves was demonstrated (Table 3, Fig. 3). Out of 150 diarrheic calves, 21 (14%) excreted *C. Parvum* oocysts in their feces, while out of 104 apparently healthy calves, only 5 (4.8%) excreted the oocysts in their feces. Statistical analysis of the results revealed that there was an association between shedding of the protozoal oocysts and diarrhea in calves ($P < 0.05$). Hall *et al.*, (1992) stated that *C. Parvum* is the second common pathogen from young calves with diarrhea and Uga *et al.*, (2000) reported that calves infected with *C. Parvum* had a higher significant rate of diarrhea than non infected calves suggesting

that *C. Parvum* is the likely cause. On the other hand, presence of *C. Parvum* oocysts in asymptomatic, non diarrheic calves constitutes a public health hazard and indicates the potential role of such animals as reservoirs of infection.

Human cryptosporidiosis is a world wide emerging zoonoses affects the gastrointestinal tract of human and persons at greatest risk are immunocompromised adults and children, especially those with AIDS, children in day care, travelers to endemic regions, dairy or cattle farm workers or contacts, household contacts of cases

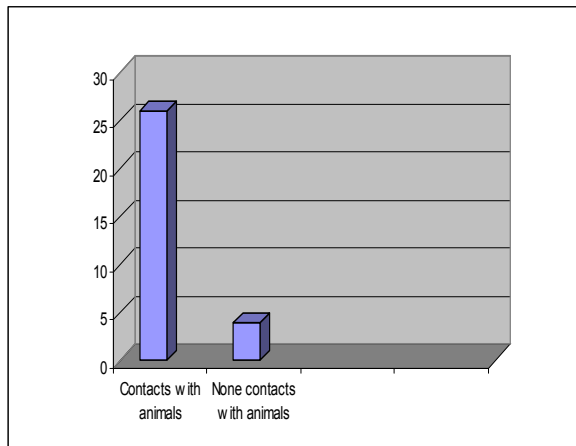


Fig. (5): The association between history of contacts with animals and infection of children with *C. parvum*.

or carriers and possibly owners of dogs or cats (Keusch *et al.*, 1992). The highest detection of *C. parvum* oocysts was observed in stool samples of the age group < 2 years old {20(20.20%)} (Table 4, Fig. 4). Statistical analysis showed high significant differences ($P < 0.01$) in detection of *C. parvum* in relation to the age groups of the examined children. These results are supported by those of Das *et al.*, (1993); Henry *et al.*, (1995) who observed that the highest detection rate of *C. parvum* was in the first two years of life in both diarrheic and control children. In Egypt, Shehata (1997) reported that out of 125 diarrheic stool samples of children below 3 years of age at Abo El-Riech pediatric hospital, *Cryptosporidium spp.* oocysts were detected in 24 (19.2%) samples, while all the 30 non-diarrheic control groups were negative for the oocysts. Table (5) and Fig. (5) represented the association between history of contacts with animals and infection of children with *C. parvum*. Among 156 and 95 stool specimens from children with history of contact with animals and non animal contacts, *C. parvum* oocysts were detected in 26 (16.66%) and 4 (4.2%) of the samples, respectively. There was a high significant difference between both groups of children in the rate of detection of the protozoal oocysts ($P < 0.01$). These results may be attributed to the fact that most of examined children belonged to the rural communities in Behera and Menoufia Governorates where they are always in close contact with farm, companion and wild animals. These results are supported by those of Miron *et al.*, (1991); Stanic-Plavlinic *et al.*, (2003).

A total of 11 random samples from diarrheic children, calves and lambs (from the same rural

area in Behera Governorate) which previously detected with MZN technique were confirmed by PCR using specific oligonucleotide primers and results recorded in Table (6). PCR detected 11 (100%) out of 11 samples with similar-sized bands (452bp) suggesting that the same genotype of *C. parvum* is present in both animal species and man in that rural area which support the evidence of zoonotic transmission. The high cost of reagents and instruments together with the need to experience which is not available in many clinical laboratories render MZN staining technique is reliable method as a screening test for detection of the cryptosporidial oocysts in stool and fecal samples from human and animals (Morgan *et al.*, 1998; Majewska *et al.*, 2000).

Giardia cysts are highly infectious for humans and infections can be established by ingestion of as few as 10 viable cysts and numerous outbreaks of giardiasis associated with contaminated water have been reported (Adam, 1991). Among 251; 254; 250 and 47 examined stool and fecal samples from children, calves, lambs and dogs, *G. Lamablia* cysts were detected in 27 (10.75%); 51 (20.08%); 63 (29.20%) and 5 (9.61%) of the samples, respectively (Table 7). Curtale *et al.*, (1998) found the protozoan cysts among school age children in Behera Governorate in much higher prevalence (24.7%). Mohamed *et al.*, (1995) described the predisposing factors for occurrence of symptomatic giardia infection among infants in rural Egypt. In addition to the age of infants, poverty, low education of parents, gender discrimination and certain environmental conditions potentiate the risk for developing the symptomatic infection.

Domestic animals living in close contact with man in rural areas may have a great opportunity to ingest cysts of *E. histolytica*. The possibility of occasional human infection from infected animals can not be discarded (WHO, 1979). Among 251; 254; 250 and 52 stool and fecal samples from the examined children, calves, lambs and dogs in different localities in Behera and Menoufia Governorates *E. histolytica* was detected in 19 (7.56%); 0; 0 and 2(3.84%) of the samples, respectively. In Egypt, Mostafa and Zaki (1992) reported a prevalence of 21.6% among children less than two years old, while Osman *et al.*, (1999) reported the frequency of *E. histolytica* infection as 5.16% among malnourished and immuno-compromised children. On the other hand, Omar *et al.*, (1978); Abou-Shady *et al.*, (1983) failed to detect *E.*

histolytica in 112 cows, 85 buffaloes, 57 sheep and 46 goats' samples in Dakahlia Governorate. Detection of *E. histolytica* in fecal samples from dogs was supported by the findings of (Grewal *et al.*, 1970; Abou-Shady *et al.*, 1983). These results emphasize the role dogs as a companion animal in transmission of *Giardia lamalia* and *E. histolytica* to man.

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دور الحيوان في نقل بعض الأوليات المعوية للإنسان في مناطق مختلفة من دلتا النيل

تم في هذه الدراسة تجميع ٨٠٧ عينة براز (٢٥١ عينة من أطفال دون سن السادسة تعاني من إسهال ، ٢٥٤ و ٢٥٠ عينة من عجول وحملان في سن ما قبل الفطام تعاني من إسهالات أو سليمة ظاهرياً ، على التوالي ، بالإضافة إلى ٥٠ عينة من براز الكلاب) من مناطق مختلفة في محافظتي البحيرة والمنوفية للكشف عن الكريبتوسبورديوم ، والجيارديا و الأميبا. تم الكشف عن الكريبتوسبورديوم باستخدام صبغة الزيل نيلسن في ٣٠ (١١,٩٥٪) ؛ ٢٦ (١٠,٢٤٪) ؛ ٣١ (١٢,٤٪) و ٢ (٣,٨٤٪) من عينات البراز من الأطفال والعجول والحملان والكلاب ، على التوالي في كلتا المحافظتين. كانت هناك علاقة قوية بين العدوى بالكريبتوسبورديوم في العجول وعمرهم وحالتهم الصحية. وقد لوحظت نفس العلاقة في الأطفال التي تم فحصها. تم تأكيد نتائج صبغة الزيل نيلسن باستخدام اختبار الإليزا والذي أثبت حساسيته في التشخيص (٩٦,٦٪).

على الرغم من ارتفاع حساسية اختبار ساسسلة تفاعلات البلمرة المتعددة مقارنةً بصبغة الزيل نيلسن في الكشف عن الكريبتوسبورديوم - وبخاصة عندما يقل عدد الحويصلات في البراز - إلا أن ارتفاع تكلفة المواد الكاشفة وعدم توافر الأجهزة اللازمة لهذا التفاعل في كل المعامل يجعل استخدام صبغة الزيل نيلسن مقبولاً.

تم أيضاً الكشف عن الجيارديا في ٢٧ (١٠,٧٥٪) ؛ ٥١ (٢٠,٠٨٪) ؛ ٦٣ (٢٩,٢٪) و ٥ (٩,٦١٪) من عينات البراز من الأطفال والعجول والحملان والكلاب ، على التوالي في كلتا المحافظتين مما يشير إلى أن كلاً من العجول والحملان والكلاب قد تمثل مصدراً هاماً لإصابة الإنسان بالجيارديا.

تم أيضاً الكشف عن طفيل الأميبا في ١٩ (٧,٥٦٪) ؛ (٠) ؛ (٠) و ٢ (٣,٨٤٪) من عينات البراز من الأطفال والعجول والحملان والكلاب ، على التوالي في كلتا المحافظتين مما يعطي للكلاب الأهمية كمصدر لعدوى الإنسان بهذا الطفيل.