EPIDEMIOLOGICAL STUDIES ON SOME ZOONOTIC ENTERIC PROTOZOA IN DIFFERENT AREAS OF NILE DELTA

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ABSTRACT

Background/Aim: This study aimed to investigate some epidemiological aspects related to the occurrence of some zoonotic enteric protozoa in different areas of Nile Delta.

Materials and methods: 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 52 fecal samples from dogs) were collected from different localities in Behera and Menoufia Governorates for detection of Cryptosporidium spp., Giardia spp., and Entamoeba histolytica.

Results: 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 to 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 to be sensitive (overall sensitivity 96.6%).

By using direct smear and formal ether method, Giardia intestinals has been detected in {27(10.76%); 51(20.08%); 63(252%)} and 5 (9.62%) of stool and fecal samples from the examined children, calves, lambs and dogs, respectively from both Governorates.

Conclusions: 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. It can be concluded that cattle, sheep and dogs are important reservoirs for cryptosporidium to man. Calves, lambs and dogs seem to be important sources for Giardia intestinals to man. Entamoeba histolytica has been detected in {19(7.56%); 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.

Keywords: Cryptosporidium, Giardia spp., Entamoeba histolytica, Calves, lambs, children

INTRODUCTION

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. 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 threatening in people who have compromised immune system. In Egypt, more than 50% of deaths among children younger than two years are due to diarrheal diseases. Although Cryptosporidium infection of livestock may have an important economic impact on farmers because of high morbidity and sometimes high rates of mortalities, the excreted cryptosporidium oocyst and Giardia cysts with feces of infected animals, particularly calves can be considered as a source of human infection. People in rural areas are also more exposed to ingest cysts of Entamoeba histolytica from infected animals.

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 for the detection of Cryptosporidium oocysts 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 test.
Eleven random fecal samples from diarrheic children, calves and lambs that had proved to be +ve for cryptosporidium by MZN technique were tested by PCR for confirmation. 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 Ul of CLB. PCR mixture: PCR buffer, 2.5 mM MgCl2, 200 mM each of (d.ATP; d.CTP and d.TTP), 2.5 U of Taq DNA polymerase.

Cryptosporidium specific Oligonucleotide primers were selected according to (10). Primer1: 5’CCGAGTTTGATCCAAAAAGTTACG AA.
Primer2: 3’TAGCTCCTCATATGCCTTGAGTA.

The mixture 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. Thirty Ul from each PCR amplificon were electrophoresed on poly acrylamide gel, stained with ethidium bromide and visualized under ultra violet transilluminator.

Statistical analysis was computed using Chi-square test $^$(3).

For detection of *Giardia intestinalis* and *Entamoeba histolytica*: Direct smear methods $^$(6) and Modified formol-ether concentration according to were performed $^$(7).

**RESULTS**

The occurrence of *Cryptosporidium parvum* in stool and fecal samples of children, calves, lambs and dogs as examined by MZN and ELISA test was shown in table 1 and figure 1. 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 (12.4%) and 2 (3.85%) samples, respectively.

Microscopically positive samples for *Cryptosporidium* were re-examined by ELISA test revealing the detection of the protozoan in 30 (11.95%); 25 (9.84%); 29 (11.6%) and 2 (3.85%) in children, calves, lambs and dogs samples, respectively. The sensitivity of ELISA in detection of the protozoan antigens in stool and fecal samples was calculated from positive samples obtained by MZN technique.

The sensitivity of ELISA reached 100; 96; 94 and 100%, respectively.

The association between occurrence of *C. parvum* infection in calves and lambs with their age was studied in table and figure (2). The examined calves were grouped according to their ages during the course of the study into three groups. *C. parvum* oocysts were detected in 20; 3 and 3 samples out of 132 (up to 1 month old); 59 (1-2 months old) and 63 (2-3 months old), respectively. In addition, *Cryptosporidium* oocysts were detected in 21 (19.63%); 7 (8.75%) and 3 (4.76%) out of 107 (up to 1 month old); 80 (1-2 month old) and 63 (2-3 months old) samples from lambs, respectively. It was obvious in both animal species that the highest occurrence of *C. parvum* infection appeared in the youngest age group (up to 1 month old). The Chi-square value (X2) was 7.234 * (P< 0.01) for the calves group. The association between occurrence of *C. Parvum* and health status of the examined calves was demonstrated in table (3) and figure (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).

Concerning human, the highest detection of *C. parvum* oocysts was observed in stool samples of the age group < 2 years old (20 (20.20%) (table 4 and figure 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. Table and figure (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 (16.67%) 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). A total of 11 random samples from diarrheic children, calves and lambs (from the same rural area in Behera Governorate) which previously proved to be positive for cryptosporidium 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). Among 251; 254; 250 and 47 examined stool and fecal samples from children, calves, lambs and dogs, *G. Lamablia* cysts were detected in 27 (10.76%); 51 (20.08%); 63 (25.2%) and 5 (9.62%) of the samples, respectively (Table 7).

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 (7.57%); 0 and 2(3.85%) of the samples, respectively (Table 8).
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Table 1: occurrence of Cryptosporidium parvum in stool and fecal samples of Children, calves, lambs and dogs in both Governorates as examined by MZN technique and ELISA

<table>
<thead>
<tr>
<th>Host</th>
<th>MZN</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve cases</td>
<td>%</td>
</tr>
<tr>
<td>Children</td>
<td>30</td>
<td>11.95</td>
</tr>
<tr>
<td>Calves</td>
<td>26</td>
<td>10.24</td>
</tr>
<tr>
<td>Lambs</td>
<td>31</td>
<td>12.4</td>
</tr>
<tr>
<td>Dogs</td>
<td>2</td>
<td>3.85</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Calves</th>
<th></th>
<th></th>
<th>Lambs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>+ve</td>
<td>%</td>
<td>No.</td>
<td>+Ve</td>
</tr>
<tr>
<td>Up to 1 month old</td>
<td>132</td>
<td>20</td>
<td>15.15</td>
<td>107</td>
<td>21</td>
</tr>
<tr>
<td>1-2 months old</td>
<td>59</td>
<td>3</td>
<td>5.08</td>
<td>80</td>
<td>7</td>
</tr>
<tr>
<td>2-3 months old</td>
<td>63</td>
<td>3</td>
<td>4.76</td>
<td>63</td>
<td>3</td>
</tr>
</tbody>
</table>

Chi-square value: $X^2 = 7.234^* \quad X^2 = 9.5^{**} \quad (P < 0.01)$

Table 3: The association between occurrence of C. Parvum and health status of the examined calves

<table>
<thead>
<tr>
<th></th>
<th>+ VE CASES</th>
<th>- VE CASES</th>
<th>TOTAL NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrheic calves</td>
<td>21</td>
<td>14</td>
<td>129</td>
</tr>
<tr>
<td>Apparently healthy calves</td>
<td>5</td>
<td>4.8</td>
<td>99</td>
</tr>
</tbody>
</table>

Chi-square value: $X^2 = 5.647^* \quad P < 0.05$

Table 4: The association between age of the examined children in both Governorates and occurrence of C. parvum in their stool

<table>
<thead>
<tr>
<th></th>
<th>+ VE CASES</th>
<th>- VE CASES</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 2 years old</td>
<td>20</td>
<td>20.20</td>
<td>79</td>
</tr>
<tr>
<td>2-4 years old</td>
<td>6</td>
<td>7.50</td>
<td>74</td>
</tr>
<tr>
<td>4-6 years old</td>
<td>4</td>
<td>5.56</td>
<td>68</td>
</tr>
</tbody>
</table>

Chi-square value: $X^2 = 10.71^{**} \quad P < 0.01$

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

<table>
<thead>
<tr>
<th></th>
<th>+ VE CASES</th>
<th>- VE CASES</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Contacts with animals</td>
<td>26</td>
<td>16.67</td>
<td>130</td>
</tr>
<tr>
<td>- None contacts with animals</td>
<td>4</td>
<td>4.2</td>
<td>91</td>
</tr>
</tbody>
</table>

Chi-square value: $X^2 = 8.51^{**}$

Table 6: Confirmed results of MZN technique by PCR for detection of C. parvum in children, calves and lambs

<table>
<thead>
<tr>
<th>Host</th>
<th>No. +ve samples as tested by MZN</th>
<th>No. +ve samples as tested by PCR</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>4</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Calves</td>
<td>4</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Lambs</td>
<td>3</td>
<td>3</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table 7: occurrence of *Giardia lamalia* infection in stool and fecal samples from children, calves, lambs and dogs

<table>
<thead>
<tr>
<th>Host</th>
<th>+ve cases</th>
<th>-ve cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Children</td>
<td>27</td>
<td>10.76</td>
</tr>
<tr>
<td>Calves</td>
<td>51</td>
<td>20.08</td>
</tr>
<tr>
<td>Lambs</td>
<td>63</td>
<td>25.2</td>
</tr>
<tr>
<td>Dogs</td>
<td>5</td>
<td>9.62</td>
</tr>
</tbody>
</table>

Table 8: occurrence of *Entamoeba histolytica* infection in stool and fecal samples from children, calves, lambs and dogs

<table>
<thead>
<tr>
<th>Host</th>
<th>+ve cases</th>
<th>-ve cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Children</td>
<td>19</td>
<td>7.57</td>
<td>232</td>
</tr>
<tr>
<td>Calves</td>
<td>0</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>Lambs</td>
<td>0</td>
<td>0</td>
<td>250</td>
</tr>
<tr>
<td>Dogs</td>
<td>2</td>
<td>3.85</td>
<td>50</td>
</tr>
</tbody>
</table>

Figure 1: Occurrence of *Cryptosporidium parvum* in stool and fecal samples of Children, calves, lambs and dogs in both Governorates

Figure 2: Association between occurrence of *C. parvum* in calves and lambs with their age
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Figure 3: The association between occurrence of *C. Parvum* and health status of the examined calves

Figure 4: The association between age of the examined children in both Governorates and occurrence of *C. parvum* in their stool

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

Hunt et al. (12) and Isaacs et al. (13) stated that MZN staining technique has been widely used as a reliable method for detection of Cryptosporidium spp. 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 other workers (13, 14, 15, 16). In spite of detection of C. parvum in higher detection rates by other researchers (17, 18). It was stated that collection of samples from diarrheic and non diarrheic calves resulted in lowering the percentage of C. parvum detection. Although, indicated moderate agreement between two diagnostic methods, with the ELISA being the more sensitive, other workers (15) showed that both methods had the same sensitivity.

It was reported that clinical infections with C. parvum in cattle are largely confined to new born calves aging (7-21 days old) (21). In addition, other workers indicated that excretion of oocysts has been found in apparently healthy cows and lambs.

It was stated that C. Parvum is the second common pathogen in young calves with diarrhea and (25), it was 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 (14). 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 zoonotic disease affecting the gastrointestinal tract of. 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 or carriers and possibly owners of dogs or cats (26). These findings results are supported by other workers (27) and (28) 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, it was 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 (29).

In the present study, it was found 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 other workers.

Since PCR confirmed detection of 11 randomly selected samples from diarrheic children, calves and lambs (previously detected by MZN technique) 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 was a reliable method for screening and detection of the cryptosporidial oocysts in stool and fecal samples from human and animals (30, 31).

Giardia cysts are highly infectious for humans and infections can be established by ingestion of as few as 10 viable cysts. Numerous outbreaks of giardiasis associated with contaminated water have been reported (32). A much higher prevalence of Giardia cysts (27.7%) was recorded among school age children in Behera Governorate in much higher prevalence (24.7%). The predisposing factors for occurrence of symptomatic Giardia infection among infants in rural Egypt (33). In addition to the age of infants, poverty, low education of parents, gender discrimination and certain environmental conditions (35).

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 neglected (5).

In Egypt, some workers (4) reported a prevalence of 21.6% among children less than two years old, while others reported the frequency of E. histolytica infection as 5.16% among malnourished and immunocompromised children. On the contrary, other workers in Egypt (37, 38) 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 other workers. These results emphasize the role of dogs as a companion animal in transmission of *Giardia lamblia* and *E. histolytica* to man.

REFERENCES


الملخص العربي

دراسة وبانية على بعض الأولويات المعوية المشتركة في مناطق مختلفة من دلتا النيل

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قسم الصحة والأمراض المشتركة، كلية الطب البيطري، جامعة الأسكندرية.

هدف هذه الدراسة أن التعرف على بعض الجوانب البيدية المرتبطة بحذف الإصابة ببعض الأولويات المعوية المشتركة في مناطق مختلفة من دلتا النيل. تم فحص عدد 807 عينة براز من (251) عينة عن الأطفال أقل من ست سنوات من العمر المصابين بالأسلاف) علاوة على (250) عينة براز من عوامل وحمالات حيوانية معدية على التوالي مصابة بالأسلاف وأخرى سلبية في مناطق مختلفة.

في محافظة المنوفية، وجدت الدراسة وجود أولويات كريبتوسورديم، الجبارديا المعوية، الأنتيمبيا هستوسولتيكا في 30، 11.95، 01٪ (12.4٪) ثم

(4٪) على التوالي من براز الأطفال والعوامل والحمال والكشوف المعوية لدى مراقبة في مناطق مختلفة. وجدت الدراسة وجود علاقة بين اصابة العقول وعمر وحالات الصحية. كما وجدت علاقة مماثلة في الأطفال المعويين مع العمر والحالات الصحية.

تم تأكيد نتائج الاختبار السابق باستخدام اختبار البذور ووجد أن حساسية الاختبار تصل إلى 97.1٪. كما وجد أن الاختبارات تفاعل السلسلة المستخدمة في أظهر حساسية أكبر في التعرف على حيوانات الطفل حتي في حال وجودها في البراز بأنثى بين الأشخاص ذوي أعراض علاوي للحمالة. فان اختبار الصبع الزيل نيلين يعتبر اختبارات معقولة من حيث الكفاءة وسرعة التشخيص وسهولتها. كما يمكن اعتبار الماشية والهجرة والحمالة خارقة للعوامل بهذا الطفل للإنسان.

تم كذلك التعرف على حيوانات طفل الجبارديا المعوية في براز الأطفال والعوامل والحمال والكشوف المعوية باستخدام اختبار الشعرية المباشر بنسبة 27 (20.8٪) (51٪) (10.4٪) على التوالي. وقد بين ذلك دور تلك الحيوانات في نقل العوامل في حيوانات الطفل للإنسان.

كما تم التعرف على بويضات طفل الأنتيمبيا هستوسولتيكا في براز الأطفال والكشوف المعوية فقط. مما يوضح دور الكشوف كمصدر للعدوى بهذا الطفل. وقد وظفت الأمية الصحية لوجود تلك الأولويات المعوية بدور الحيوانات كمصدر للعدوى وأهمية اتباع الطرق الصحية في رعاية الحيوانات لتقليل اصابة الإنسان بتلك الأولويات.