Molecular and Conventional Detection of Zoonotic *Giardia* and *Cryptosporidium* in Children and Calves in Upper Egypt

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**Abstract** *Giardia* and *Cryptosporidium* are wide spread pathogens of human and many species of mammals. This study aimed to investigate the potential direct transmission of *Giardia* and *Cryptosporidium* between cattle calves and the surrounding living children and to improve the knowledge of zoonotic *Giardiasis* and *cryptosporidiosis* situation in Assiut Governorate, Egypt. Faecal samples from 70 diarrheic children (above 5 years) and 62 diarrheic calves (1-3 months) were collected from the same villages at Assiut Governorate, Egypt. Samples were subjected to conventional microscopic examination for *Giardia* and *Cryptosporidium*. Positive samples were subjected to molecular identification. By conventional microscopic examination, *Giardia* cysts were detected in 27 out of 70 (38.57%) child’s stool samples, while *Cryptosporidium* oocyst were detected in 12 samples (17.14%). Mixed infection of *Giardia* and *Cryptosporidium* were detected in all *Cryptosporidium* positive samples. In children, 81.48% of *Giardia* samples were *G. intestinalis* assemblage B meanwhile, all samples were negative by PCR for *C. parvum*. In calves 29.03% of faecal samples were positive for *Giardia* by microscopic examination, while only 12.90% of those samples were positive for *Cryptosporidium* oocysts. 21 out of 26 (80.76%) *Giardia* positive samples were positive to *G. intestinalis* assemblage B and 22 out of 26 *Cryptosporidium* positive samples (84.61%) were positive for *C. parvum*. High prevalence of *G. intestinalis* assemblage B infection detected among children was significantly associated with contact with calves explaining the existence of its zoonotic transmission and more studies are needed to investigate the zoonotic potential of *Cryptosporidium* in the study area.

**Keywords:** *Giardia*, *Cryptosporidium*, Zoonoses, PCR, children, Calves


1. Introduction

Gastrointestinal protozoon parasites are major health problem with a high prevalence worldwide [1]. In Egypt, parasites are considered to be the main etiologic agent of diarrhea, with prevalence reaching 61% [11,19,20]. The prevalence of intestinal protozoa infection was 57.6% in patients with gastrointestinal troubles [10].

*Giardiasis* is a major diarrheal disease was found throughout the world. In most mammals, giardiasis is caused by *Giardia intestinalis*. The rate of human infection with *G. intestinalis* in Egypt varies between 10 and 34.6% [4,11,14,16]. The most frequent genotype of *G. intestinalis* is the assemblage B (80%) [14].

Research on *G. intestinalis* in livestock, particularly cattle, has shown that the parasite is very common in this population and tends to infect younger calves leading to high prevalence of infection within herds [21-26]. Calves have been reported to be infected with *G. intestinalis* as early as four days of age and have the highest intensity of cyst excretion (10²-10⁶ cysts/gram) between the ages of 4-12 weeks [22]. Because dairy cattle can shed high levels of *G. intestinalis* and inhabit watershed areas, there has been much concern about the potential risk of zoonotic *Giardia* infections in human populations. *G. intestinalis* is etiological agent for diarrheal disease in cattle by itself but is often linked to another common intestinal parasite, *Cryptosporidium* [5-24]. *Cryptosporidium* spp. are similar to *Giardia* in terms of clinical signs, host range, zoonotic potential, and modes of transmission. Many studies have demonstrated concurrent *G. intestinalis* and *Cryptosporidium* spp. infections in dairy calves [7-15].

*Cryptosporidium* spp. infection is a leading cause of diarrhea in Egypt. *Cryptosporidium* spp. prevalence among individuals with diarrhea in Egypt reaches up to 49% [12,17,19-28]. The zoonotic potential for cryptosporidiosis has been proven in farmers and their farm animals infected with *C. parvum* [13]. A high prevalence of *C. parvum* was reported among individuals with diarrhea (31.1%) [19]. Most cases of human cryptosporidiosis are due to infections with the human species *C. hominis* or the zoonotic *C. parvum* [9]. In animals, *Cryptosporidium* spp.
infection implies both an economic loss and a significant source for zoonotic infection. A Cryptosporidium prevalence of 32.2% has been reported in ruminants [17]. In particular, a high infection rate (30.2%) of Cryptosporidium oocyst was detected in the faecal specimens of neonatal calves [2].

In Egypt, like other developing countries, the risk of zoonotic infection related to animals is high due to keeping of livestock and pets inside houses in most rural areas and absence of public education about the risk of zoonotic diseases transmitted from animals [28].

In rural villages of Egypt, there are many smallholder cattle farms consisting of 1–5 animals for milk and draft purposes. Young calves are considered as a reservoir for these parasites, and transmission of Giardia and Cryptosporidium from cattle to cattle handlers has been suggested in Egypt.

The present report aimed to improve the knowledge of Giardiasis and cryptosporidiosis situation in Assiut, and to investigate potential direct transmission of Giardia and Cryptosporidium between cattle calves and their surrounding living children.

2. Materials and Methods

Faecal samples were collected from 62 diarrheic calves ranged from 1-3 months of age, those calves from different villages of Assiut Governorate during field visits to these villages. Stool samples were collected from 70 children above 5 years of age from the same villages complaining from diarrhea. Diarrhea was defined as at least three loose stools within the last 24 h. Each study participant had to provide one stool sample on the day of the field visit. Faecal samples were collected from September, 2013 to August 2014.

Faecal samples were collected rectally from each animal. Stool samples from children and calves were immediately placed in dry, clean, leak proof plastic disposable cups with lids labeled with name, age, date and sex of the child and with age, date and sex of animal. Stool samples were stored on frozen cold packs until transported to the laboratories. Once at the laboratories, part of the samples was stored at 4°C until conventional microscopic examination and the other part were stored at -20°C for molecular examination of positive samples.

2.1. Conventional Microscopic Methods

Each stool specimen was concentrated using formalin-ethyl acetate sedimentation and zinc sulfate flotation concentration methods. Direct microscopic examination of saline, iodine wet mount preparations of stool specimens were prepared to screen for Giardia cysts and/or trophozoites. Smears from each stool samples were stained using modified Ziehl-Neelsen staining technique to detect Cryptosporidium oocysts in the faeces. Parasites were identified on the basis of cysts and/or trophozoites and oocysts color, shape, and contents [29].

2.2. Molecular Methods

All child’s stool samples positive for Giardia and Cryptosporidium oocyst by conventional examination were subjected to molecular examination in order to know if the detected protozoan parasites were the zoonotic type or not. Also all calves’ faecal samples positive for Giardia plus those positive for Cryptosporidium oocyst by conventional examination were subjected to molecular examination. The genomic DNA was extracted from calf’s and child’s stool samples using DNA extraction-kit (SBS Gentech, China) according to the instructions of the manufacturer and then used for polymerase chain reaction (PCR) amplification.

Giardia detection: primers used for detection of Giardia intestinalis assemblage B were F-tpiB (forward) 5’-AATAGCAGCACARAAACGTGTATCTG-3’ and R-tpiB (reverse) 5’-CCCCATGTCACAGCATCT-3’, which give segments at 81-bp tpi specific for assemblage B [6], PCR conditions were done as previously described by Cacciò et al. [8], Read et al. [23]- Bertrand et al. [6].

Cryptosporidium detection: the primer set described by Laberge et al. [18] was used for detection of Cryptosporidium parvum. Sequence of primers were as follow: forward 5’-GCCCATGTCCAGCATCTTTC-3’ and reverse 5’-TCCCTCTCTCTAGTACCAACAGGA-3’.

The size of the amplified product was 358 bp, PCR conditions were done as previously described by Laberge et al. [18]. The sizes of the DNA amplicons were determined by a 1.0% agarose gel electrophoresis, ethidium bromide staining, and ultraviolet trans illumination compared to a 1-kb DNA ladder run concurrently in the same gel slab in both techniques. Negative control samples were double distilled water. Positive control samples were, Giardia intestinalis cysts “Human isolate H-3, aka CH-3” and Cryptosporidium parvum “Iowa isolate” oocysts (CpI) of bovine source both were purchased from Waterborne TM (P102M, Waterborne TM, USA).

2.3. Data Analysis Procedures

Data were collected, tabulated statistically analyzed using SPSS 20.0 software. Categorical variables were described by number and percent.

2.4. Ethical Consideration

The study was approved by the ethics committee of the Faculty of Medicine, Assiut University. All participants were informed about the study’s purpose and procedures. Written informed consent was obtained from the parental or guardian authorities on behalf of the study children prior to study enrolment.

3. Results

3.1. Children

Giardia cysts were detected in 27 out of 70 (38.57%) stool samples by microscopic examination, while Cryptosporidium oocyst were detected in 12 samples (17.14%) as shown in Table 1. Those 27 Giardia positive samples included the 12 Cryptosporidium positive ones (mixed infection). Using PCR identification as shown in Table 2, of those positive samples 81.48% were positive to G. intestinalis assemblage B (Figure 1). Meanwhile, all samples were negative by PCR to C. parvum (Figure 2).
3.2. Calves

29.03% of faecal samples were positive for *Giardia* by microscopic examination, while only 12.90% of those samples were positive for *Cryptosporidium* oocyst (Table 1).

![Figure 1](image)

**Figure 1.** PCR products of stool samples for detection of *Giardia intestinalis* assemblage B as determined by 1% agarose gel electrophoresis. M: 50 bp ladder, Lane 1, 3, 5-8 positive samples, Lane 2&4 negative samples lane + is the positive control and lane - is the negative control

![Figure 2](image)

**Figure 2.** PCR products of stool samples for detection of *Cryptosporidium parvum* as determined by 1% agarose gel electrophoresis. M: 100 bp ladder, Lane 1-10 positive samples, lane + is the positive control and lane - is the negative control

When all the 18 positive samples for *Giardia* plus the 8 positive samples for *Cryptosporidium* (*n* =18+8= 26) were subjected to molecular examination revealed 21 out of 26 (80.76%) were positive to *G. intestinalis* assemblage B (Figure 1), and 22 out of them (84.61%) were positive for *C. parvum* (Table 2; Figure 2).

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<thead>
<tr>
<th>Protozoan parasite</th>
<th>Children</th>
<th>Calves</th>
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<tbody>
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<td><em>Giardia</em> species</td>
<td>Examined samples</td>
<td>Positive samples</td>
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<td>70</td>
<td>27</td>
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<tr>
<td><em>Cryptosporidium</em> species</td>
<td>70</td>
<td>12</td>
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<tr>
<td><em>Giardia intestinalis</em> (assemblage B)</td>
<td>Examined samples</td>
<td>Positive samples</td>
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<tr>
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<td>27</td>
<td>22</td>
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<tr>
<td><em>Cryptosporidium parvum</em></td>
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4. Discussion

Parasitic zoonoses caused by helminths and protozoa are endemic in Egypt. Intestinal infections of parasitic zoonoses are exceedingly common and widespread and are a leading cause of diarrhea, particularly among children in rural areas. *Giardia* and *Cryptosporidium* are important causes of diarrhea in Egypt. The high prevalence of both parasites in humans and cattle in rural areas in Egypt, and the use of contaminated surface and drinking water in Assiut by animal faeces and human sewage suggested a potential for zoonotic transmission. So our aim was to detect the zoonotic *Giardia intestinalis* assemblage B and *Cryptosporidium parvum* in children and calves in Assiut villages by the conventional and molecular methods.

*Giardia* cysts were detected in 38.57% of child’s stool samples by microscopic examination, this was agreed with El-Naggar et al. [11], Foronda et al. [14], Helmy et al. [17] and Baiomy et al. [4], who stated that the rate of human infection with *G. intestinalis* in Egypt varied between 10 to 34.6%. Results of molecular examination revealed that 81.48% of those samples were positive to *G. intestinalis* assemblage B, which indicate that most of the infected children with *G. intestinalis* were infected with the zoonotic type which is usually transmitted from infected animals to man. This results coincided with Soliman et al. [25] who found that the most frequent genotype of *G. intestinalis* in Egypt was assemblage B and the possibility that humans infected through contact with infected cows through a zoonotic route. Also this result agreed with Amer [3] who stated that assemblage B was the predominant assemblage in the human, and stressed the significance of neonatal calves as reservoir of zoonotic *Giardia* infections in human. The zoonotic transmission from infected cattle may be supported by the detection of *G. intestinalis* cyst in 29.03% of calf’s faecal samples by microscopic examination, and 80.76% of them were positive to *G. intestinalis* assemblage B. This indicate that assemblage B was prevalent in Assiut villages and transmitted from infected calves to children in contact with them.

Regarding *Cryptosporidium* species oocyst were detected in 17.14% of child’s stool samples by microscopical examination, this result coincided with El Shazly et al. [12], Youssef et al. [27], Mousa et al. [19] and Helmy et al. [17] who stated that prevalence of *Cryptosporidium* among individuals in Egypt ranged from 0-49%. Surprisingly all these samples were negative by PCR for detection of *C. parvum*, the zoonotic type, which means those cases were infected with another species of *Cryptosporidium*. This result came in contrast with almost all the studies done before in Egypt, which usually detect...
C. parvum in individuals lived in rural areas and in contact with infected cattle calves. For example, El-Sherbini and Mohammad [13] concluded that the zoonotic potential for cryptosporidiosis has been proven in farmers and their farm animals infected with C. parvum. Mousa et al. [19] found high prevalence of C. parvum among individuals with diarrhea in Egypt (31.1%). But this finding may be slightly similar to the finding of Helmy et al. [17] who found that human Cryptosporidium prevalence was 49.1%, and only 1.2% of them was C. parvum. These findings may indicate that infection cycles in human and cattle in the study area are largely separated, and the risk of zoonotic infection emanating from cattle is negligible. It may be also explained by the low prevalence of cryptosporidiosis in cattle calves in the study area.

In calves, only 12.90% of faecal samples were positive microscopically for Cryptosporidium oocyst, and 84.61% of them were positive for C. parvum by molecular examination. This prevalence was lower than Amer et al. [2] and Helmy et al. [17] who found the prevalence of Cryptosporidium oocysts in ruminants in Egypt is 30.2% and 32.2% respectively by microscopical examination.

5. Conclusions

G. intestinalis and Cryptosporidium species were prevalent among calves and children in rural villages of Assiut Governorate. G. intestinalis assemblage B was the most prevalent which indicate the zoonotic transmission between animals and human in the area. Meanwhile, C. parvum was not detected in the examined individuals, and identified in low prevalence in the examined calves. this finding may need more studies to confirm if there is a possible transmission of C. parvum between animals and human or not in the study area.

References