Prevalence and Genotyping of Cryptosporidium spp. in Farm Animals in Egypt

Magdy Elsayed MAHFOUZ1), Nabila MIRA1) and Said AMER1)*

1)Department of Zoology, Faculty of Science, Kafrelsheikh University, Kafr El Sheikh, 33516, Egypt

(Received 27 May 2014/Accepted 7 August 2014/Published online in J-STAGE 2 September 2014)

ABSTRACT. In this study, we examined the prevalence and molecular characteristics of Cryptosporidium in buffalo, dairy cattle and sheep in different farms at Kaf El Sheikh Province, Egypt. Rectal fecal samples, including 466 samples from buffalo, 1697 from cattle and 120 from sheep, were collected from different ages and screened by modified Ziehl-Neelsen acid-fast microscopy for detection of Cryptosporidium oocysts. All studied farms were positives with an overall prevalence of 1.29% in buffalo (4.17% in calves versus 0.48% in adults), 7.07% in cattle (6.90% in calves versus 10.20% and 6.10% in heifers and adults, respectively) and 2.50% in sheep (4.40% in lambs versus 1.30% in adults). PCR-RFLP analyses of small-subunit rRNA genes from positive specimens revealed the occurrence of C. parvum and C. ryanae in buffalo; C. parvum, C. ryanae, C. bovis and C. andersoni in cattle and only C. xiaoi in sheep. Genotypes distribution showed that C. ryanae was the dominant species (60.0%) followed by C. parvum (40.0%) in buffalo calves. Meanwhile, in cattle calves, C. parvum was the commonest species (74.23%) followed by C. ryanae (16.10%) and C. bovis (9.70%). Subtyping of C. parvum based on sequence analysis of the polymorphic 60 kDa glycoprotein gene locus showed the presence of subtypes IIdA20G1 and IIaA15G1R1 in both buffalo and cattle calves, addressing the potential role of calves in zoonotic cryptosporidiosis in Egypt.

KEY WORDS: Cryptosporidium, Egypt, genotyping, subtyping, zoonosis

MATERIALS AND METHODS

Collection of specimens and examination: Samples of this study were collected during June 2010–November 2011 from 5 private farms designated A, B, C, D and E, which were situated at different localities in Kafr El Sheikh (130 km north of Cairo), Egypt (Table 1). In cattle and buffalo farms, the neonates were kept in separate pens with milk feeding of suckling calves performed manually, allowing no direct contact of calves with their dams. Based on animals’ age, the examined cattle and buffalo were classified into 3 groups: less than 3 months of age (calves), 4–24 months (heifers) and older than 2 years (adults). A total of 2,163 fecal samples were collected from 570 calves including 450 cattle and 120 buffalo, 462 heifers comprising 323 cattle and 139 buffalo and 1,131 adults with 924 cattle and 207 buffalo. Additionally, 120 sheep samples (45 neonates and 75 adults) were sampled. Fecal specimens were collected directly from the rectum of each animal with a gloved hand and transferred into plastic cups labeled with the ear tag number and farm name. The fecal samples were screened for Cryptosporidium oocysts by microscopy using the modified Ziehl-Neelsen stain [10]. Positive specimens were stored in 2.50% potassium dichromate with distilled water by centrifugation. Oocysts were detected microscopically in 5 out of 120 (4.17%) fecal samples from calves and in one out of 207 of adults (0.48%), while no oocysts were detected in the 139 examined specimens from heifers. Nonetheless, the infection rate in cattle was higher than that detected in buffalo animals, addressing the effect of animal species on the infection rate, especially in case of older animals. A total of 31 out of 450 (6.90%) fecal samples from calves, 33 out of 323 (10.20%) from heifers and 56 out of 924 (6.10%) from adult cattle proved to have Cryptosporidium oocysts. Microscopic examination of sheep samples revealed the occurrence of Cryptosporidium oocysts in 2 out of 45 lambs (4.40%) and only one sample out of 75 adult sheep (1.30%).

Cryptosporidium infection: Cryptosporidium oocysts were detected in all examined farms in spite of species or age of the subjected animals (Table 1). In water buffalo, Cryptosporidium oocysts were detected microscopically in 5 out of 120 (4.17%) fecal samples from calves and in one out of 207 of adults (0.48%), while no oocysts were detected in the 139 examined specimens from heifers. Nonetheless, the infection rate in cattle was higher than that detected in buffalo animals, addressing the effect of animal species on the Cryptosporidium infection rate, especially in case of older animals. A total of 31 out of 450 (6.90%) fecal samples from calves, 33 out of 323 (10.20%) from heifers and 56 out of 924 (6.10%) from adult cattle proved to have Cryptosporidium oocysts. Microscopic examination of sheep samples revealed the occurrence of Cryptosporidium oocysts in 2 out of 45 lambs (4.40%) and only one sample out of 75 adult sheep (1.30%).

Cryptosporidium species: RFLP analysis of PCR products of the SSU rRNA gene indicated the presence of C. ryanae in 4 specimens (66.67%, 3 calves and one adult) and C. parvum in 2 specimens (33.33%) of buffalo samples. In addition, results revealed that 3 Cryptosporidium species were detected in cattle calves with varying prevalence; C. parvum (23/31; 74.23%), C. ryanae (5/31; 16.10%) and C. bovis (3/31; 9.70%), indicating the dominance of C. parvum. On the other hand, in heifers and adults, C. andersoni was the overwhelming species accompanied with a minor fraction.

Table 1. Infection rates of Cryptosporidium in different age groups of buffalo, cattle and sheep in Kafr El Sheikh Province, Egypt, based on acid fast staining-microscopy

<table>
<thead>
<tr>
<th>Farm Name</th>
<th>Farm animals</th>
<th>Sample size</th>
<th>No. positive</th>
<th>Infection rate (%)</th>
<th>Sample size</th>
<th>No. positive</th>
<th>Infection rate (%)</th>
<th>Sample size</th>
<th>No. positive</th>
<th>Infection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A C</td>
<td>Cattle</td>
<td>154</td>
<td>4</td>
<td>2.60</td>
<td>123</td>
<td>2</td>
<td>1.60</td>
<td>370</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>Farm B C</td>
<td>Cattle</td>
<td>159</td>
<td>16</td>
<td>10.10</td>
<td>123</td>
<td>13</td>
<td>10.60</td>
<td>318</td>
<td>21</td>
<td>6.60</td>
</tr>
<tr>
<td>Farm C C</td>
<td>Cattle</td>
<td>137</td>
<td>11</td>
<td>8.0</td>
<td>75</td>
<td>18</td>
<td>24.0</td>
<td>236</td>
<td>33</td>
<td>14.0</td>
</tr>
<tr>
<td>Farm D B</td>
<td>Buffalo</td>
<td>57</td>
<td>1</td>
<td>1.80</td>
<td>89</td>
<td>0</td>
<td>0.0</td>
<td>116</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Farm E B</td>
<td>Buffalo</td>
<td>63</td>
<td>4</td>
<td>6.40</td>
<td>50</td>
<td>0</td>
<td>0.0</td>
<td>91</td>
<td>1</td>
<td>1.10</td>
</tr>
<tr>
<td>Farm C S</td>
<td>Sheep</td>
<td>45</td>
<td>2</td>
<td>4.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>75</td>
<td>1</td>
<td>1.30</td>
</tr>
</tbody>
</table>

RESULTS

Cryptosporidium infection: Cryptosporidium oocysts were detected in all examined farms in spite of species or age of the subjected animals (Table 1). In water buffalo, Cryptosporidium oocysts were detected microscopically in 5 out of 120 (4.17%) fecal samples from calves and in one out of 207 of adults (0.48%), while no oocysts were detected in the 139 examined specimens from heifers. Nonetheless, the infection rate in cattle was higher than that detected in buffalo animals, addressing the effect of animal species on the Cryptosporidium infection rate, especially in case of older animals. A total of 31 out of 450 (6.90%) fecal samples from calves, 33 out of 323 (10.20%) from heifers and 56 out of 924 (6.10%) from adult cattle proved to have Cryptosporidium oocysts. Microscopic examination of sheep samples revealed the occurrence of Cryptosporidium oocysts in 2 out of 45 lambs (4.40%) and only one sample out of 75 adult sheep (1.30%).

Cryptosporidium species: RFLP analysis of PCR products of the SSU rRNA gene indicated the presence of C. ryanae in 4 specimens (66.67%, 3 calves and one adult) and C. parvum in 2 specimens (33.33%) of buffalo samples. In addition, results revealed that 3 Cryptosporidium species were detected in cattle calves with varying prevalence; C. parvum (23/31; 74.23%), C. ryanae (5/31; 16.10%) and C. bovis (3/31; 9.70%), indicating the dominance of C. parvum. On the other hand, in heifers and adults, C. andersoni was the overwhelming species accompanied with a minor fraction.
of *C. parvum* (2/89; 2.30%). In sheep, *C. xiao* was the only detected species in the 3 positively infected animals. Based on the RFLP patterns, no mixed infections in one animal were detected in the present study.

DNA sequencing of representative samples confirmed the identification of the *Cryptosporidium* species. Blasting the obtained sequences with those in database and the deduced phylogenetic analysis (Fig. 1) demonstrated that out of all the isolated samples, the sequences of *C. parvum* were 100% identical to each other in spite of the difference in host species and age of the animals. Moreover, these sequences were identical to reference sequences reported from *C. parvum* (AB513856). Meanwhile, specimens of *C. andersoni* generated one sequence that was identical to those from China (KF271477), Japan (AB089285) and Egypt (AB513856).

*C. ryanae* specimens generated two sequence types. The first was derived from two isolates of buffalo and was identical to reference sequences reported from buffalo in Brazil, Egypt and India (JX59848, AB712388 and JQ002562, respectively). The second sequence type was generated from 3 specimens of cattle and was identical to that derived from dairy calves in China (HQ179574) as well as from cattle in Egypt (AB777174), while it has one base pair difference (99.0% homology) to sequences JX515551 isolated from dairy calf and KF128757 isolated from yak in China.

One type of sequence was obtained from *C. bovis* that was identical to the reference reported from Egypt (AB777172) and 99.0% similarity, with one base pair difference, compared to the sequences described from yak in China (KF128742).

Fig. 1. Phylogenetic relationship of *Cryptosporidium* spp. based on sequences of the partial SSU rRNA gene. Evolutionary relationships of 43 taxa were inferred using the Neighbor-Joining method [39] with the coccidian *Eimeria tenella* (AF026388) as an out-group. Numbers at the internodes correspond to percent bootstrap values from 2,000 replicates. Egyptian isolates are bolded.
from cattle in Japan (AB746197) and from humans in Australia (JQ362488).

C. xiaoi from sheep generated one sequence type that was identical to sequences from sheep in U.S.A. and China (FJ896044 and KF907825). In addition, 99.0% similarity, one base pair difference, was noticed when compared to sequences from goat in Spain and sheep in Romania (GU553016 and JX258864, respectively).

C. parvum subtypes: In the current study, sequence analysis of C. parvum isolates on the gp60 gene locus revealed no difference among subtypes isolated from buffalo or cattle and generated two types of sequences. The first includes 3 sequences and belongs to the subtype IId (IIdA20G1 subtype). This sequence type was identical to sequences obtained from dairy cattle and buffalo in Egypt (AB514086, AB514089 and AB712389) and 99.0% with sequences with those reported from children in Kuwait and Sweden (AY738186 and JQ028866). The second type includes 2 sequences and belongs to IIA (IaA15G1R1 subtype). These sequences were identical to those reported from humans in Kuwait [42], Slovenia [41] and Australia [47] as well as from dairy calves in the Czech Republic [25], dairy and buffalo calves from Egypt [4, 5] and dairy calves and humans from Egypt [21].

DISCUSSION

The coccidian protozoa Cryptosporidium infects a wide range of livestock, resulting in significant economic loss and public health concern. In the present study, the infection rate varies according to the age and species of the host. Cryptosporidium oocysts were identified microscopically in 4.17% fecal samples of water buffalo calves and in 0.48% of adults, while no oocysts were detected in specimens from heifers. The infection rate in calves reported herein is much lower than that detected in buffalos, addressing the effect of animal species in pre-weaned calves differs from a country to another and from a study to others, reflecting the profound effect of the environment and husbandry regimen on the infection rate. An exclusive presence of C. parvum was the prevailing along with a small fraction of with C. parvum (2.30%). It is evident that the distribution of Cryptosporidium species in pre-weaned calves differs from a country to another and from a study to others, reflecting the profound effect of the environment and husbandry regimen on the infection rate. An exclusive presence of C. parvum, or with a minor fraction of other cryptosporidia, in pre-weaned dairy calves was previously reported in many countries [3, 44], contrasting the predominance of C. bovis and C. ryanae in some other countries [17, 29]. In the present study, C. andersoni was reported in a number of Asian countries, such as China [28, 49], as well as European countries [33]. In the United States and Canada, C. andersoni occurs in adult cattle beside different proportions of C. bovis and C. ryanae [8, 15]. Although C. andersoni represents no public health concerns, it can cause impairment of weight gain in growing animals in case of heifers (10.20%). These results are comparable to those previously reported in dairy cattle in Egypt; 30.20% [3] and 13.60% [5]. In the present study, high differences were detected in the infection rates between different farms; 2.60% versus 10.10% in calves, 1.60% versus 24.0% in heifers and 0.54% versus 14.0% in adults, in low and high rate infected farms, respectively. These differences reflect the effect of farm related factors on the incidence of Cryptosporidium. The management regimen in the farm A imposes very restricting roles on the in and out of the animals, and the sucking calves up to the ~6 weeks of age are reared in ground separated pens with very limited opportunity of calves to contact with the discharged fecal matters. Because farms B and C belong to the same body, the move of animals between the two farms allows introducing new fecal members to the herds. The calves are reared in ground pens, which, in turn afford high possibility to contact with the fecal matter. This might be a factor behind the high infection rates in both farms.

Microscopic examination of sheep samples revealed the occurrence of Cryptosporidium oocysts in 4.40% of lambs and 1.30% of adult sheep. Similar results were reported in sheep from Brazil [18] and Egypt [31]. However, this infection rate is remarkably lower than that reported in sheep and lambs in Norway (15–24.0%), Romania (13.70%), Brazil (25.0%) and Australia (16.90%) [23, 34, 37, 53], addressing the habitat on the incidence of the disease.

Cryptosporidium species: RFLP and sequence analyses of SSU rRNA gene fragment (Table 2) indicated the presence of C. ryanae (66.67%) and C. parvum (33.33%) in buffalo samples. Similar results were reported in water buffalo from Egypt [4]. On the other hand, Caccio et al. [9] and Gomez-Couso et al. [20] reported only C. parvum in water buffalo calves in Spain and Italy, respectively. Feng et al. [17] reported only C. ryanae in buffalo calves from Nepal. These discrepancies may be attributed to farm, breed and/or climatic factors.

C. parvum was the dominant prevailed species in cattle calves (74.23%) versus C. ryanae (16.10%) and C. bovis (9.70%), whereas, in heifers and adults, C. andersoni was the prevailing along with a small fraction of with C. parvum (2.30%). It was reported in a number of Asian countries, such as China [28, 49], as well as European countries [33]. In the United States and Canada, C. andersoni occurs in adult cattle beside different proportions of C. bovis and C. ryanae [8, 15]. Although C. andersoni represents no public health concerns, it can cause impairment of weight gain in growing animals.
and milk production in dairy cows and thus reduced feed efficiency [12].

In concordance with results reported herein, C. xiaoi was the dominant Cryptosporidium species in ewes and lambs in China [54] and in sheep in Australia [53]. In contrast, Imre et al. [24] reported the dominance of the zoonotic C. parvum in new born lambs in Romania, while C. cervine was the major genotype in adult sheep in China [48]. The possible occurrence of periparturient transmission of Cryptosporidium spp., especially C. xiaoi, from ewes to lambs was addressed for cycling of the infection [54]. In spite of the limitation of sample size, the obtained results indicated that Cryptosporidium in sheep has no communal health concerns. Generally, the high identity of the generated sequences of similar type may be due to the semi-conservative nature of the SSU rRNA gene locus [52] common origin of the infection and/or the possibility that the infection was due to epidemic clone (the genetic fitness of this Cryptosporidium strain) [2, 7]. Moreover, geographic differences in the distribution of specific alleles may exist.

C. parvum subtypes: Occurrence of IIa and IId subtypes highlights the public health risk posed by Cryptosporidium parvum recovered in the present study. The incidence of C. parvum subtype families differs widely according to geographic locations and hosts. Subtype IIa was reported as the predominant subtype family of C. parvum in cattle in Egypt, Malaysia and China [3, 29, 50], sheep and goats in Spain [35] and human in Kuwait, Jordan, Iran and Egypt [21, 22, 42, 43] in addition to HIV/AIDS patients in Malaysia [27]. However, it was detected at low frequencies in cattle in Europe and is largely absent in cattle and humans in U.S.A. and Canada [51]. Meanwhile, IId is the most prevalent subtype family of C. parvum in calves worldwide, especially in industrialized countries [19, 25], implicating the cattle as the main source of human IId infections.

In conclusion, Cryptosporidium oocysts were detected in all of the examined farms in spite of species or age of animals, which signify the widespread of the infection in Egypt. In cattle, the infection rates were higher than that detected in buffalo or sheep, revealing the effect of animal species on Cryptosporidium infection rate, especially in case of older animals. The common occurrence of the C. parvum IId and IId subtype families in both buffalo and dairy calves in Egypt highlights the potential role of Cryptosporidium zoonotic in these animals.

**ETHICS STATEMENT.** Fecal samples were obtained with consent from farm owners of subjected animals. The study was approved by the Institutional Committee of the Post Graduate studies and research at Kafr El Sheikh University, Egypt. All efforts were made to minimize animal suffering. No experimentation was done on animals.

**ACKNOWLEDGMENTS.** This study was partially support by Kafr el sheikh University. Thanks to farm owners for approval and facilities provided during collection of samples. Thanks extended to Dr. Abd El Naby Tahoon and Dr. Ehab El Sebaay, National Institute of Animal Health, Kafr El Sheikh Provincial lab, for their immense help.

**REFERENCES**

1574

bubalis). Vet. Parasitol. 150: 146–149. [Medline] [CrossRef]


33. Ondrácková, Z., Kváč, M., Sak, B., Kvetonová, D. and Rost, M. 2009. Prevalence and molecular characterization of Cryptosporidium spp. in dairy cattle in South Bohemia, the Czech Republic. Vet. Parasitol. 165: 141–144. [Medline] [CrossRef]


