ABSTRACT

Cryptosporidium is a ubiquitous zoonotic protozoan parasite which is associated with diarrhea in humans and animals. Despite the importance of buffalo farming in Iran, little is known in this country about the abundance and distribution of Cryptosporidium spp. in the animal species. The present study was designed to investigate the prevalence of Cryptosporidium oocysts in river buffalo calves of Khuzestan province, in the southwest of Iran. Rectal fecal specimens from 90 calves in different age groups were screened microscopically for Cryptosporidium oocysts using modified Ziehl-Neelsen stain. Among all animals sampled, 41 calves were positive (45.5%). Rates and intensity of the infection were significantly higher in calves less than 2 months of age (66.6%) compared to other age groups. There was also no significant difference between the prevalence rate of infection in males and females. Cryptosporidium infection was associated with the occurrence of diarrhea in these calves. Considering the pervasive occurrence and negative effects of the infection on the health condition and the growth performance of buffalo calves, infections should receive increased attention by both farmers and veterinarians.

Keywords: river buffaloes, buffalo calves, Cryptosporidium spp., oocysts, diarrhea, Khuzestan, Iran

INTRODUCTION

Cryptosporidium spp. (Apicomplexa: Cryptosporidiidae) are among the most important coccidian parasites of mammals, birds, reptiles and fish, and are distributed worldwide. The high morbidity that is associated with high mortality rate in some instances, makes Cryptosporidium infection of great economic concern for farm animals (Casemore et al., 1997). Cryptosporidiosis is more severe in newborn animals and causes severe diarrhea that is sometimes accompanied with anorexia, reduced milk intake, dehydration, growth retardation, stiffness, hyperpnoea, slow gait and depression (Fayer, 2004). Although the adult animals are generally refractory to infection, infected animals can act as asymptomatic carriers and shed large numbers of oocysts into the environment and remain a main source of infection to other domestic and wild animals (Xiao et al., 1993). Cryptosporidium causes an emerging zoonotic disease and has considered as an important cause of chronic life-threatening and cholera-like diarrhea in HIV infected patients. Calves are an important source of human cryptosporidial infections (Olson et al., 2004). Presently, the increasing populations of immune-compromised
persons and various outbreaks through infection by Cryptosporidium oocysts have placed an even greater emphasis on this pathogen. This situation has triggered efforts towards the investigation on the parasite (Juranek, 1995). However, there is no information on the prevalence and importance of these parasites in Iranian buffalo herds. According to the latest available statistics, there are about 459 thousand head of buffaloes in Iran, which is ranked 16th among 43 countries in the world. There is a considerable population (>138,000 head) of river buffalo (Bubalus bubalis) in Khuzestan province in the southwestern region of Iran which play a significant role in rural life by producing milk and meat while tolerating the impact of harsh environmental conditions (Taheri Dezfuli et al., 2011). The buffalo is characterized by outstanding features such as high percentage of fat, rapid weight gain, high relative strength against some diseases, optimal use of low-quality food and its use in producing some special products such as mozzarella cheese. In Khuzestan province, rearing and breeding practices on buffalo farms is under the rural system and the animals are reared in open areas due to the long hot season of the region. The relative importance of buffalo production in various regions of the province is the same and buffaloes are primarily reared for milk productions such that about 40 percent of dairy products of the province are allocated to the buffaloes. Totally, the buffalo population in the province and the quality and value of its products make the buffalo an important native animal in this region and necessitate that more attention be paid to it (Naderfard and Qanemy, 1997). In Iran, there are no published data available on Cryptosporidium infection in river buffalo calves. Since calves have been found to be more susceptible to coccidian parasite infections, the objective of this study was to obtain information about the prevalence of Cryptosporidium amongst the river buffalo calves in farms of Khuzestan, a southwestern province of Iran.

**MATERIALS AND METHODS**

The study was conducted in Khuzestan, a southwestern province of Iran, from December 2011 to July 2012. Khuzestan province has a border of about 64,236 km², between 47 degrees and 41 minutes to 50 degrees and 39 minutes of eastern longitude from the prime meridian and 29 degrees and 58 minutes to 33 degrees and 4 minutes of northern latitude from the equator (Statistical Book of Khuzestan province, 2006). The province has hot and wet summers, a mild spring and cold winters. The buffalo population mainly comprises local domestic species, which are well adapted to the climate of the area. A total of 90 fecal samples (37 female and 53 male) were collected from calves (birth to 1 year old) from four different localities of Khuzestan province. These calves were grouped into four different age groups: Group A, < 2 months (n=15), Group B, 2-4 months (n=27), Group C, 4-6 months (n=34) and Group D, > 6 months (n=24). The farms participating in this study had been selected randomly. On each farm, individual samples were collected from three to four randomly selected calves. The farms included in this survey were visited once. The age of the animals was based on owner records. Fecal samples were collected directly from rectum or immediately after defection in a wide-mouth plastic bottle. All the samples were classified according to their consistency, as normal or diarrheic feces (liquid or semi-liquid feces). Until tested for Cryptosporidium spp. oocysts in the laboratory, the fecal samples were stored under refrigerated
conditions. The samples were processed by water–ether concentration (Bukhari and Smith, 1995) and stained by a modified Ziehl-Neelsen’s acid-fast method (Casemore et al., 1985). Slides were examined using a routine light microscope by oil immersion objective (x1000). The positive samples were further studied by micrometry method to measure the mean of the oocyst diameter. The intensity of infection was assessed and graded by counting the cryptosporidial oocysts according to Anderson and Bulgin (1981); as slight (1–5 oocysts/field), moderate (6–20 oocysts/field), and severe (more than 20 oocysts/field). Fischer exact and chi-square tests were used to compare infection rates among different age groups and between sexes. A p value of <0.05 was considered statistically significant.

**RESULTS**

Fecal samples were collected from 90 calves. On all farms, there were calves that shed *Cryptosporidium* spp. in their feces. Among all animals sampled, 41 calves were positive (45.5%). Infection rates were significantly (p< 0.05) higher in calves less than 2 months of age (66.6%) than in the remaining age groups. Also there was a significantly higher infection intensity in the first age group (Table 1). The prevalence rate of the infection according to the age groups was as follows:

- Group A- Ten of 15 examined calves (66.6%) were infected, among which two showed slight, two moderate and six severe infection.
- Group B- Nine of 27 (33.3%) were infected, among which three showed slight, five moderate and one severe infection.
- Group C- Thirteen of 34 calves (38.23%) were infected, among which seven showed slight, four moderate and two severe infection.
- Group D- Nine of 24 calves (37.5%) were infected, among which two showed slight, five moderate and two severe infection.

The mean of oocyst diameter was 4.56±0.65 μm with a range of 4.20-5.70 μm (n=100). The size and shape of the oocysts identified in positive samples were consistent with that of *C. parvum* (Fayer, 2000) (Figure 1). Microscopic examination showed that the calves of both sexes were infected with *Cryptosporidium*: 45.28% (24/53) of the male and 45.94% (17/37) of the female animals were infected. There was no significant difference between these prevalences (p> 0.05).

All 90 samples of known fecal consistency, either with or without oocysts, were used to determine the relationship between *Cryptosporidium* infection and diarrhea. Out of 90 samples, 41 (45.5%) were positive and 49 (64.4%) were negative for *Cryptosporidium* oocysts. While 39.02% (16/41) of the oocyst positive samples were diarrheic, only 22.44% (11/49) of the oocyst negative samples were diarrheic (P< 0.05).

**DISCUSSION**

Little is known of the prevalence of buffalo cryptosporidiosis in Iran for comparison purposes. However, studies on bovine cryptosporidiosis have shown prevalence rates from 1.61% to 14.59% in other regions in Iran (Azami, 2007). In the Latium region of central Italy, 14.7% of buffalo calves were found to have antigens of *C. parvum* (Rinaldi et al., 2007). Studies carried out in calves in Poland have reported a prevalence of 25% (Pilarczyk and Balicka-Ramisz, 2002). In the United States, 35% of calves were reported as
shedding oocysts (Santín et al., 2004). In Germany, 19–36% of cattle (Joachim et al., 2003) and 93% of cattle in the Japan (Uga et al., 2000) were positive. In the present study, there was evidence that, in Khuzestan, a southwestern province of Iran, calves are infected by Cryptosporidium spp., indicating that buffalo cryptosporidiosis is endemic and locally widespread. The overall prevalence of 45.5% implies the presence of the disease in the majority of the herds in the study area whereas studies on bovine cryptosporidiosis have shown prevalence rates from 1.61% to 14.59% in Iran (Azami, 2007). The variation between the different prevalence rates of the disease may be attributed to the system of rearing and management in addition to the level of hygienic measures applied. The hot and humid climate of Khuzestan province is advantageous to both the expansion of river buffalo herds and the high prevalence of parasites in these animals. These environmental conditions may have contributed optimal conditions for oocyst maintenance and thus, for buffalo infection. Other factors like poor nutrition, poor sanitation, and overcrowding increased the level of infection and incidence of the disease due to stress-induced immunosuppression. Some studies have shown that parasite oocysts are able to survive for extended periods in faeces and the environment, and very low doses of viable oocysts can cause an infection (Chako et al., 2010). Previous studies showed that a strong association was observed between housing system and risk of Cryptosporidium infection (EL-Khodery and Osman, 2008). In this study animals were reared under a closed type of housing system with a non-cemented floor and they were fed on the ground. Feeding of calves on the ground increases the chances of contamination of the feed with Cryptosporidium oocysts and a non-cemented floor is difficult to clean. Sex of the calf was not associated with Cryptosporidium spp. oocyst shedding in this study, and this is in agreement with the report of Silverlås et al. (2009). The prevalence rate in the young age group (66.6%) showed a statistically significant difference from other older calves (P

![Figure 1. Oocysts of Cryptosporidium spp. in modified Ziehl-Neelsen staining in buffalo calves feces (X1000).](image)
< 0.05). This finding was at variance with earlier reports (Maldonado-Camargo et al., 1998; Gow and Waldner, 2006) where such age range was concluded to have no role in cryptosporidiosis epidemiology. The occurrence of high infection rates in this age category is attributed to poor immunity and ease of oocyst contamination through bucket-feeding (Castro-Hermida et al., 2005). Many previous studies have shown a significant association between diarrhea and shedding (Trotz-Williams et al., 2005; Castro-Hermida et al., 2006; Geurden et al., 2006). In the current study, infection rates were statistically significantly higher in diarrheic calves. Thus, it can be concluded that Cryptosporidium infection is associated with the occurrence of diarrhea in these calves. Other workers have also reported a statistically significant association between cryptosporidial infection and diarrhea (Pilarczyk and Balicka-Ramisz, 2002; Santín et al., 2004; Joachim et al., 2003; Uga et al., 2003).

Table 1. The prevalence rate of Cryptosporidium infection in buffalo calves according to different age groups and infection intensity.

<table>
<thead>
<tr>
<th>Age</th>
<th>Total</th>
<th>Intensity</th>
<th>Infected</th>
<th>Uninfected</th>
<th>Prevalence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>Slight</td>
<td>2</td>
<td>5</td>
<td>66.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>27</td>
<td>Slight</td>
<td>3</td>
<td>18</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>34</td>
<td>Slight</td>
<td>7</td>
<td>23</td>
<td>38.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>24</td>
<td>Slight</td>
<td>2</td>
<td>13</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td></td>
<td>41</td>
<td>49</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Table 2. The prevalence rate of Cryptosporidium infection in buffalo calves according to the sexes.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>Infected</th>
<th>Non-infected</th>
<th>Prevalence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>53</td>
<td>24</td>
<td>29</td>
<td>45.28</td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
<td>17</td>
<td>20</td>
<td>45.94</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>41</td>
<td>49</td>
<td>45.5</td>
</tr>
</tbody>
</table>
However, Snodgrass et al. (1986) and Kaminjolo et al. (1993) found no statistically significant association between infection and diarrhea, although cryptosporidia were detected more frequently in diarrheic than in healthy calves (Snodgrass et al., 1986; Kaminjolo et al., 1993). Measures to control and prevent Cryptosporidium infection should be considered in animal production facilities, both to increase productivity of the animals and also to reduce the risk of human infection. As a conclusion, in this study area, clinical cryptosporidiosis cases exist and this area is highly contaminated with Cryptosporidium spp. Therefore they represent an important starting point for further studies aimed to the genetic characterization of the isolates for refining their host range, transmission dynamics and zoonotic potential.

ACKNOWLEDGMENT

This study was supported by a research grant provided by Shahid Chamran University of Ahvaz.

REFERENCES


Buffalo Bulletin (December 2014) Vol.33 No.4


