

Prevalence of *Cryptosporidium* spp. in calves under one year old in Ilam county (Iran), from March 2014 to February 2015

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Type of article: Original**Abstract**

Introduction: Cryptosporidium is a coccidian parasite that can infect most mammals, including humans. This parasite is an important cause of neonatal diarrhea and morbidity in calves, causing substantial economic loss to animal husbandry. Because of the importance of this parasite in calves, this study was designed in order to determine prevalence of Cryptosporidium spp. among calves under one year old in Ilam county (Iran).

Methods: Prevalence of Cryptosporidium spp. was estimated using a cross-sectional study. Fecal samples from 400 calves under 1 year old were collected in Ilam County, from March 2014 to February 2015 and they were microscopically analyzed using modified Ziehl-Neelsen method (mZN).

Results: The overall prevalence of Cryptosporidiosis in calves was 16% (64/400). According to age categories, the highest infection was observed in calves under one month old, and the lowest infection occurred in calves 6 to 12 months old. Age was significantly associated with Cryptosporidium spp. infection ($p < 0.05$). The prevalence of Cryptosporidiosis in diarrheic and non-diarrheic calves was 27.3 and 12.4 % respectively, which indicated a significant association between positivity and diarrhea ($p < 0.05$). The highest prevalence was observed in winter followed by autumn, spring and summer respectively. No static difference was found between prevalence of Cryptosporidiosis and seasons of sampling. Also, calves' gender was not associated with Cryptosporidium spp. infection in this study.

Conclusion: Our results provide useful information about the distribution of Cryptosporidium spp. Also, the role of potential risk factors including age, sex, clinical status and seasons of sampling was indicated in studied calves from Ilam County.

Keywords: *Cryptosporidium* spp., prevalence, Calves, Ilam, Iran

1. Introduction

The protozoan *Cryptosporidium*, belongs to the phylum Apicomplexa, the class Sporozoa, the subclass Coccidiasina, the order Eucoccidiorida, the suborder Eimeriorina and the family Cryptosporidiidae. Humans, birds and livestock such as cattle, sheep, pigs, goats and horses, are susceptible to infection of the various species of *Cryptosporidium* parasite (1). *Cryptosporidium* spp. can develop and proliferate in the microvillus borders of the enteric epithelium cells. *Cryptosporidium* is also capable of infecting other tissues, such as the respiratory and renal epithelia, particularly in immunocompromised humans (2, 3). Cryptosporidiosis in cattle and humans is mainly caused by *C. parvum* (4). Other species that infect cattle are as follow: *C. andersoni*, *C. bovis* and *C. ryanae* (3, 5). *C. parvum* is an important species that infects gastrointestinal epithelium of most mammalian hosts, this species causes severe acute diarrheal enteritis with substantial morbidity and mortality among young calves, children and immunocompromised individuals such as AIDS patients (6). Contact with infected calves has been implicated as the cause of Cryptosporidiosis in humans (7). So, determination of prevalence and risk factors of infection with *Cryptosporidium* spp., are very important. So far, many studies have been conducted to determine incidence rate of Cryptosporidiosis in calves in different parts of the world (8, 9). Nevertheless, a few studies have been designed on a wide scale and in large numbers of calves especially in the west of Iran (10, 11). In this investigation, we determined

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the prevalence of Cryptosporidiosis in 400 studied calves under one year old in Ilam County, using microscopic examination. Moreover, different potential risk factors of Cryptosporidiosis were investigated.

2. Material and Methods

2.1. Research design and sampling procedure

A cross-sectional epidemiological study was conducted on calves in Ilam County (Iran), from March 2014 to February 2015. Four hundred fecal samples were collected from random dairy and family farms. Fecal samples were collected directly from the rectum of the animals. Calves were classified according to the age categories: pre-weaned calves aged 1-15 days (n=58) 16-30 days (n=82), weaned calves aged 31-60 days (n=72), juvenile calves aged 61 to 180 days (n=88) and calves aged 181 to 365 days (n=101). Also information about the studied calves such as their age, gender, clinical status of and season of sampling were recorded.

2.2. Microscopic examination

Fecal samples were concentrated by using zinc sulfate flotation technique as previously described (12). 200 µl of floated supernatant of each samples were collected and in order to detect *Cryptosporidium* oocysts, 20 µl of each floated supernatant were fixed on a slide and stained using modified Ziehl-Neelsen (mZN) staining method and examined under bright field microscopy.

2.3. Statistical analysis

Data were analyzed using the SPSS version 16 (SPSS Inc., Chicago, Illinois, USA), prevalence and 95% confidence interval (CI) were determined. Analysis for the association between risk factors and *Cryptosporidium* infection was performed using the Chi-square test. Differences were considered statistically significant when p-value was less than 0.05.

3. Results

In this study, the overall prevalence of *Cryptosporidium spp.*, in calves was 16% (64 of 400 samples) by modified acid-fast staining technique (mZN). The prevalence of *Cryptosporidium spp.* infection varied by age group, ranging from 6.9% to 31%. Age was found to be the significant risk factor for *Cryptosporidium* infection in this study. Calves <1 months old were more likely to be infected with *Cryptosporidium spp.* than calves >1 months old (p<0.05). According to our age groups, the highest rate of infection was observed in age group 1 followed by age group 2, 3, 4 and age group 5, respectively (Table 1). Out of the 95 samples from calves with diarrhea and 305 samples without diarrhea, 26 (27.3%) and 38 (12.4%) samples were found positive, respectively (Table 2). This indicated a significant association between positivity and diarrhea (p<0.05). Cryptosporidiosis percent prevalence recorded in female calves was little higher than male prevalence (Table 2). On statistical analysis by Chi-square test, non-significant difference was found between calves' gender and *Cryptosporidium* infection (p>0.05). The highest prevalence of Cryptosporidiosis was observed in the winter season followed by autumn, spring and summer seasons by incidence rate 21%, 18%, 14% and 11%, respectively (Table 1). No significant statistical differentiation was found between seasons of sampling and prevalence of *Cryptosporidium spp.* infection (p>0.05).

Table 1. Prevalence of Cryptosporidiosis in studied calves according to their age groups and seasons of sampling data are presented in number of infected (n) and percent (%).

Age	Spring		Summer		Autumn		Winter			
Age groups (day)	n	%	n	%	n	%	n	%		
1-15	18	31	6	40	4	28.5	4	25	8	44.4
16-30	20	24	3	17.6	5	27.7	6	42	7	36.8
31-60	9	12.6	2	14.2	1	4.5	2	10.5	2	12.5
61-180	10	11.3	2	7.1	1	5.2	4	16	3	11.5
181-365	7	6.9	1	3.8	-	0	2	7.6	1	4.7
Total	64	16	14	14	11	11	18	18	21	21

Table 2. Risk factors that are predicted to be associated with *Cryptosporidium* infection in studied calves.

Factor	n	Positive; n (%)	
Gender	Male	182	27 (14.8)
	Female	218	37 (16.9)
Clinical status	Diarrheic	95	28 (27.3)
	None diarrheic	305	36 (12.4)

4. Discussion

The gastrointestinal infection Cryptosporidiosis, which is caused from infection with *Cryptosporidium spp.*, is an important and well-recognized zoonosis (9). In our study, results based on finding oocysts by mZN staining indicated that *Cryptosporidium spp.* is a common parasite of calves in Ilam County, west of Iran, with an overall prevalence of 16% (64/400). There are a few studies which have been done regarding prevalence rate of Cryptosporidiosis in calves in Iran. In the study that was carried out by Mahami et al. in Ilam, 3.6% (8/227) of studied cattle were infected with *Cryptosporidium spp.* (10). Similar studies have been done in other regions of Iran, Azami reported 6.25% (30/480) of studied cattle in Isfahan province were infected by *Cryptosporidium spp.* In Kerman province, 63 of 291 dairy calves (21.65%) were identified positive using the mZN staining technique (13). Razmi et al. reported that 28.3% of 300 pre-weaned calves were infected by *Cryptosporidium spp.*, using mZN method (14). Many studies have been conducted to determine prevalence of *Cryptosporidium spp.* in calves and other hosts in other countries. In Iraq, 32 of 220 (14.5%) neonatal calves were positive using capture direct ELISA method (15). In England, the prevalence rate in cattle was 10.2%, using IFA test, moreover the highest prevalence rate was observed in calves under 1 month old (16). Santin et al. reported that, from 971 calves, 345 calves (35.5%) were infected with *Cryptosporidium spp.* using IFA and PCR methods. Furthermore, higher prevalence rate was detected in pre-weaned calves (253 out of 503; 50.3%) compared with post-weaned calves (92 out of 468; 19.7%) (17). In addition, 35.7% of cattle in Vietnam were positive for *Cryptosporidium spp.*, using PCR method (1). Several factors such as breed, husbandry and management system, age, nursing conditions of the calves, seasons and other factors can be responsible for the differences in the prevalence of *Cryptosporidium spp.* between calves in different parts of the world (18, 19, 20). In the present study, a significant difference was found between prevalence rate of *Cryptosporidium spp.* and calves' age. Also, prevalence of Cryptosporidiosis in diarrheic calves was significantly higher than non-diarrheic calves. In the current study, the highest prevalence of Cryptosporidiosis was found in calves aged 1 to 15 days (31%) and the lowest in animals aged 6 to 12 months (6.9%). The prevalence of Cryptosporidiosis in calves with 1 to 12 months old was significantly lower than calves with 1 to 30 days old. Decreasing prevalence of *Cryptosporidium spp.* infection with age in cattle has been constantly observed (1, 13, 16, 17, 21-24). In this study, *Cryptosporidium spp.* infection was observed in both healthy and diarrheic calves, there was significant association between the occurrence of diarrhea and Cryptosporidiosis infection, which comes in agreement with previous studies (1, 25, 26). The highest season-wise percent prevalence was observed during winter (21%) followed by autumn (18%), spring (14%) and the lowest in summer (11%). However, the difference between seasons was not statistically significant. Many studies have been conducted regarding potential risk factors for *Cryptosporidium* infection in calves and in these studies, different results have been obtained about effects of season on the prevalence of the Cryptosporidiosis in calves. Mohammed et al. have reported that the prevalence of Cryptosporidiosis in calves and cattle has been higher in winter (27). On the other hand, it has been reported that a particular season has had no effect on prevalence of the infection in calves (28, 29). In our study, the highest number of cases occurred in the seasons of winter and autumn when the rainfall is highest. Similar observations were seen in Brazil and Indonesia. In Kuwait, high prevalence was found in the cool months of November to April, when rainfall is marginal (30). In our survey, there is no significant relationship between Cryptosporidiosis and calves' gender. This result is confirmed with different studies (17, 31). However, Nouri and Toroghi, reported a higher infection in male diarrheic calves than in female calves (32, 33).

5. Limitations of the study

This study had some limitations. All fecal samples were first concentrated and then stained by mZN method, although sensitivity and specificity of this method is high the samples with low intensity infection may be considered negative. Another limitation was that the sampling was only done in Ilam County, so the results may not reflect the situation in other regions of Ilam province.

6. Conclusions

Our results showed that the prevalence of Cryptosporidiosis in studied calves was 16%. Moreover, from studied risk factors, age and clinical status were found to be the significant risk factors for *Cryptosporidium* infection. This study provides useful information about the distribution of *Cryptosporidium spp.* infection according to different risk factors such as: age, sex, clinical status and seasons of sampling in calf populations in Ilam County, west of Iran. We hope the results of our study will be useful for veterinary and public health in our country.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

Both authors contributed to this project and article equally. Both authors read and approved the final manuscript.

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