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ORIGINAL ARTICLE

Prevalence of *Cryptosporidium* spp. infection in some dairy herds of Mashhad (Iran) and its association with diarrhea in newborn calves

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Abstract A 1:1 matched case-control study of calves less than 1 month of age was carried out by weekly visits to some dairy farms in Mashhad, Iran. Fecal samples were collected over a 6-month period from a total of 112 calves with clinical signs of diarrhea and from 112 matched animals without clinical signs of diarrhea as assessed by a scoring system. The samples were investigated for the presence of *Cryptosporidium* spp. oocysts by modified Ziehl–Neelson staining method. *Cryptosporidium* spp. oocysts were present in 51.8% of calves with diarrhea and in 21.4% of the controls. Among diarrheic calves, the highest prevalence of *Cryptosporidium* oocyst shedding was in age group 8–14 days (74.5%), and the lowest prevalence of *Cryptosporidium* oocyst shedding was in age group 22–30 days (23.8%). The McNemar test in all sampled calves showed significant differences in odds ratio for the presence of *Cryptosporidium* spp. in fecal samples, and the excretion of parasite in the feces of scouring calves was significantly higher (odds ratio 6.1) than in healthy calves. Differences between animals with and without diarrhea were statistically significant for age groups 1–7 and also 8–14 day-old calves. These results indicate that in these industrial dairy farms in Mashhad, infections by *Cryptosporidium* should be considered as a potential cause for newborn calf.

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Introduction

Cryptosporidium is a protozoan genus that belongs to the phylum Apicomplexa, class Sporozoasida, subclass Coccidiasina, order Eucoccidiorida, suborder Eimeriorina and family Cryptosporidiidae (Sterling and Arrowood 1993). This parasite has been detected in a wide range of vertebrate hosts. Infection, which usually causes self-limiting diarrhea in human and animals, can be fatal in immunocompromised individuals (O'Donoghue 1995; Fayer et al. 1997; Caccio and Pozio 2006). This parasite causes significant neonatal morbidity in cattle, causing weight loss and delayed growth, which leads to large economic losses (Xiao et al. 1999; McDonald 2000). Infection in cattle is highly age-dependent. Young calves show the highest prevalence of infection and shed the organism with highest intensity (Garber et al. 1994; Quílez et al. 1996).

In particular, cattle are infected with at least four *Cryptosporidium* species, including *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium andersoni*, and the *Cryptosporidium* deer-like genotype (Fayer et al. 2006; Feng et al. 2007); however, pre-weaned calves have been reported to be predominantly infected with the zoonotic species, *C. parvum.* The infection with this species in post-weaned calves and older animals is almost completely replaced with *C. andersoni*, *C. bovis*, and the deer-like genotype (Santín et al. 2004; Fayer et al. 2006; Thompson et al. 2007).

C. parvum has been incriminated as an important cause of neonatal diarrhea in calves and most often affects calves under 1 month of age. The affected calves may shed large

Table 1 Some features of dairy farms in this study

Farms	Average number of total population per month during the study period	Average number of milking cow per month during the study period	Average number of calves under 1month age per month during the study period (male–female)	Number of sampled pairs
1	1,439	515	23–24	18
2	1,333	451	20–22	13
3	1,606	568	26–28	29
4	1,567	504	18–20	11
5	635	182	10–10	15
6	407	157	11–13	5
7	1,149	405	22–16	21

numbers of infective oocysts in the feces (Nydam et al. 2001). *Cryptosporidium* infection of calves may be asymptomatic or causes clinical signs ranging from mild intermittent diarrhea to profuse watery diarrhea with concomitant dehydration (Fayer and Ungar 1986). Clinical symptoms depend on the parasite species involved and specific host characteristics, such as age and immunological state (Xiao et al. 2004).

The zoonotic potential of infection with *Cryptosporidium* has been largely studied. Genotyping of the parasite has shown that human and other animals can be infected with the same species of *Cryptosporidium* (Monis and Thompson 2003). Calves are an important source of human cryptosporidial infections (Olson et al. 1997).

Since there was no documented information on the status of cryptosporidial infection and its association with diarrhea among newborn calves in dairy farms around Mashhad (Iran), we devised a matched case-control study to investigate the relationship between diarrhea and excretion of *Cryptosporidium* spp. oocyst in feces and also to determine the prevalence of *Cryptosporidium* infection in newborn calves with diarrhea in the area.

Materials and methods

Study population

The study was performed in the Northeast region of Iran, in the area of Mashhad, the capital city of the Khorasan Razavi province. The city is located at 36.20° latitude and 59.35° East longitude, in the valley of the Kashaf River near Turkmenistan, between the two mountain ranges of Binalood and Hezar-masjed. The city benefits from the proximity of the mountains, having very cold winters and pleasant springs. Mashhad has more than 500 dairy farms with an estimated 70,000 cows of mostly Holstein/Friesian breed.

In the present study, seven industrial dairy herds were selected. We carried out a cross-sectional study, with a random cluster sampling design, from May 2008 to October 2008 (Table 1). The cow breed in all these farms was Holstein/Friesian. In all farms, calves were separated from their dams after they received colostrum and were housed in individual pens until weaning at 3 months of age.

Sampling technique

All farms were visited weekly and fecal samples were collected. Upon each visit, all calves under 1 month of age with signs of diarrhea were sampled, provided that they had not received prior treatment with antibiotics. Fecal consistency was scored on a 4-point scale as described by Larson et al (1977). For this study, a score of 3 or 4 indicated the presence, and a score of 1 or 2 the absence of diarrhea.

For each case calf, a corresponding calf with normal feces (with a fecal score of 1 or 2), closest in age (with a maximum age difference of 4 days) on the same farm was sampled as control. Whenever an animal was sampled, a form was completed with related information. Sample size was calculated as the minimum number of calves necessary

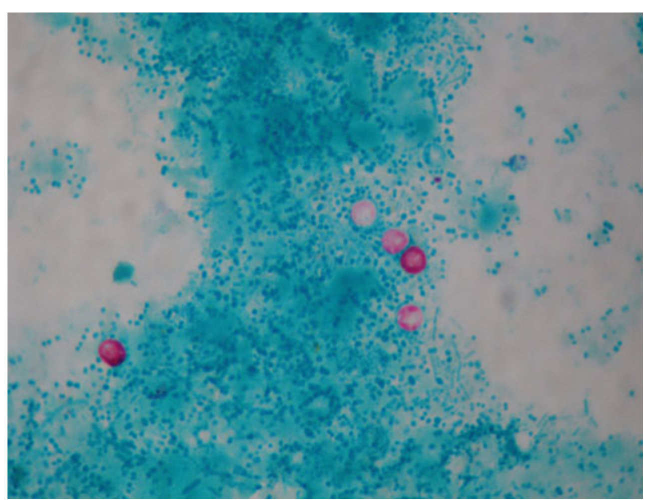

Fig. 1 *Cryptosporidium* spp. oocysts stained by modified Ziehl–Neelson method

Table 2 Frequency of *Cryptosporidium* spp. infection in different farms

Farms	*Cryptosporidium*		Total
	Positive (%)	Negative (%)	
1	14 (38.9)	22 (61.1)	36
2	8 (30.8)	18 (69.2)	26
3	27 (46.6)	31 (53.4)	58
4	8 (36.4)	14 (63.6)	22
5	12 (40)	18 (60)	30
6	3 (30)	7 (70)	10
7	10 (23.8)	32 (76.2)	42
Total	82 (36.6)	142 (63.4)	224

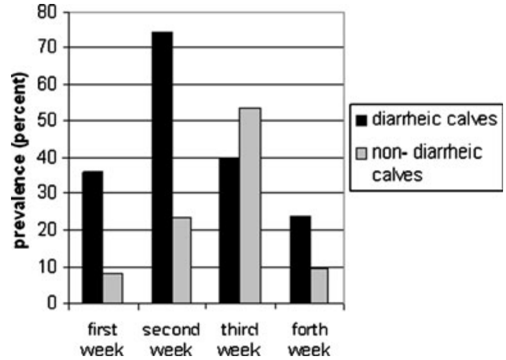

Fig. 2 Prevalence of *Cryptosporidium* spp. infection in different age groups of diarrheic and non-diarrheic calves

to detect a difference between 36.66% (prevalence rate of *Cryptosporidium* in calves with diarrhea; Radfar et al. 2006) and 19.92% (prevalence rate of *Cryptosporidium* in calves without diarrhea; Radfar et al. 2006) at a confidence level of 95% and a power of 80% (Thrusfield 2005); 105 calves with diarrhea and 105 controls were needed. We sampled 112 pairs of calves, each pair consisting of a diarrheic calf and a non-diarrheic calf.

Controls were discarded whenever they turned into cases within 1 week of sampling and, consequently, the match was not used in the analysis.

Fecal samples were collected directly from the rectum by stimulating the anus with fingers. All samples were transported on ice to the parasitology laboratory of the School of Veterinary Medicine of Ferdowsi University of Mashhad on the same day. Samples were stored at 4°C until processing.

Parasitological examination

Moderately thick fecal smears were prepared and air-dried. After fixation by methanol, the fecal smears were stained by modified Ziehl–Neelson staining. The stained smears were observed under microscope with a ×400 to ×1,000 magnification. The *Cryptosporidium* spp. oocysts were visualized as bright red round bodies against a pale green background, containing elongated naked sporozoites (Fig. 1). Cryptosporidium infection was scored positive or negative based on the presence or absence of the oocyst in examined microscopical fields in each sample.

A sample was considered positive for *Cryptosporidium* spp. if an oocyst was detected with the correct morphology, i.e., optical properties, internal structure, size, and shape as described by Fayer (1997).

Statistical analysis

The McNemar test was used to analyze these 1:1 individually matched case-control data and to estimate the associations between diarrhea and the presence of *Cryptosporidium* spp. oocysts in fecal samples. Data were analyzed with SAS statistical software (Version 9.1) and P values less than .05 were considered to be statistically significant.

Results

Descriptive statistics

Fecal samples were collected from 112 pairs of calves; in each pair, one calf was diarrheic and another one was non-diarrheic.

Table 3 Association (odds ratio) between diarrhea and *Cryptosporidium* infection in calves originating from some industrial dairy herds of Mashhad, Iran

Calves	Number of pairs with case+control +	Number of pairs with case+control −	Number of pairs with case−control +	Number of pairs with case−control−	Total number of pairs	Odds ratio	95% CI/ odds ratio	*p* values
1–7 days old	1	8	1	15	25	8	1.07–354.9	0.0455[a]
8–14 days old	9	30	2	10	51	15	3.8–129.5	0.0001[a]
15–21 days old	5	1	3	6	15	0.3	0–4.1	0.6171
22–30 days old	1	4	1	15	21	4	0.3–196.9	0.3711
All calves	16	43	7	46	112	6.1	2.7–16.1	0.0001[a]

[a] Statistically significant

In all farms, there were calves that shed *Cryptosporidium* spp. in their feces (Table 2). The average age of a scouring calf was 13.08 days (median 11 days with a range of 1 to 30 days) and for control animals, 13.58 days (median 12 with a range of 2 to 30 days). Among all animals sampled, 82 calves were positive. *Cryptosporidium* infection was detected in 58 out of 112 (51.8%) diarrheic and 24 (21.4%) out of 112 non-diarrheic calves.

Distribution of matched pairs (each pair consists of a case and a control) in different age groups of newborn calves is shown in Table 3.

Cryptosporidium infection was detected in nine out of 25 (36%), 38 out of 51 (74.5%), six out of 15 (40%), and five out of 21(23.8%) of 112 diarrheic animals in age groups 1–7, 8–14, 15–21, and 22–30 days old, respectively.

Therefore, the highest risk of being infected with *Cryptosporidium* spp. in diarrheic calves occurred in the second week of life. Among non-diarrheic calves, *Cryptosporidium* infection was detected in 8%, 23.5%, 53.3%, and 9.5% in age groups 1–7, 8–14, 15–21, and 22–30 days old, respectively (Fig. 2).

Analytical statistics

Comparison between non-scouring and scouring calves using the McNemar test in all sampled calves showed significant differences in odds ratio for the presence of *Cryptosporidium* spp. in fecal samples. The excretion of parasite in the feces of diarrheic calves was significantly higher (odds ratio 6.1) than in healthy calves (Table 3).

Differences between animals with and without diarrhea were statistically significant for age groups 1–7 and also 8–14 day-old calves and odds ratios were 8 and 15, respectively (Table 3).

Discussion

In the present study, infection with *Cryptosporidium* spp. in neonatal calves was prevalent and was also associated with clinical diarrhea.

The detection rate of *Cryptosporidium* spp. in newborn diarrheic calves in this study (51.8%) is higher than those found in neonatal diarrheic calves in other parts of Iran (Radfar et al. 2006) and also in other countries (from 14.1% to 44.4%; Reynolds et al. 1986; Snodgrass et al. 1986; Moore and Zeman 1991), but similar to that reported by De la Fuente et al. (1999) (52.3%) in calves up to 30 days old in Spain.

The detection rates of *Cryptosporidium* infection in different age groups of diarrheic animals are generally in accordance with the pattern of *Cryptosporidium* oocyst shedding reported for calves (Tzipori et al. 1983; De la Fuente et al. 1999). The highest prevalence of *Cryptosporidium* oocyst shedding was in age group 8–14 days (74.5%), thus the highest risk of being infected with *Cryptosporidium* in diarrheic calves occurred in the second week of life and the lowest rate of *Cryptosporidium* oocyst shedding was in age group 22–30 days (23.8%).

Calves infected with *C. parvum* usually develop diarrhea in 72 to 96 h. Therefore, the high detection rate in age group 1–7 days (36%) indicates that most of these calves were infected immediately after birth.

There was a significant association between *Cryptosporidium* spp. infection and diarrhea (calves shedding oocysts had 6.1 times the risk of being diarrheic as uninfected calves).

Many previous studies have shown a significant association between diarrhea and shedding (Quılez et al. 1996; Wade et al. 2000; Trotz-Williams et al. 2005; Maddox-Hyttel et al. 2006; Castro-Hermida et al. 2006; Geurden et al. 2006; Singh et al. 2006). However, these studies did not match each diarrheic animal with an animal as control (non-diarrheic).

In this study, each animal was matched with a normal animal with the closest age. This method helps to prevent confounding factors (Thrusfield 2005). In addition, calves in our study were all under 1 month of age and were therefore within the age group known to show the highest prevalence of *C. parvum* infection and diarrhea.

From work conducted elsewhere, it is well known that this parasite is one of the pathogens most commonly found in scouring calves and that it may be detected either alone or with other enteropathogens (Bulgin et al. 1982; Reynolds et al. 1986; de la Fuente et al. 1999).

Conclusion

Our results indicate that, in studied industrial dairy farms of Mashhad, infections by *Cryptosporidium* should be considered as one of the causes of calf neonatal diarrhea and also as a differential diagnosis when investigating the etiology of scouring in calves under 1 month of age by veterinarians who work on buiatrics.

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