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Prevalence of *Cryptosporidium* spp. in farmed animals from steppe and high plateau regions in Algeria

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Abstract. This study was conducted to investigate the prevalence of *Cryptosporidium* spp. and associated potential risk factors in farmed animals from different steppe and high plateau regions in Algeria. A total of 289, 254 and 149 faecal samples of cattle, sheep and dromedary camels respectively, and tracheas of 135 broiler chickens were screened for the presence of *Cryptosporidium* spp. by formalin-ether concentration method and modified Ziehl-Neelsen staining. Overall, *Cryptosporidium* spp. was detected in 36.7%, 15%, 8.9% and 2% of examined cattle, sheep, broiler chickens and dromedary camels. In cattle, the highest prevalence was observed in the neonatal calves (52.6%) and the presence of *Cryptosporidium* spp. was significantly associated with diarrhoea. Ovine cryptosporidiosis was found in more of 80% of sampled farms and lambs aged between 1-6 months (20.3%), followed by neonatal lambs (18.7%) were the most infected. *Cryptosporidium* excretion in sheep was not associated with presence of diarrhoea. The presence of cryptosporidia in broiler chickens showed a higher rate in birds aged of 16-24 days (30%) than in those of 35-44 days (3.5%). None of broiler chickens more than 44 days was found to be positive for *Cryptosporidium*. *Cryptosporidium* in dromedary camels was reported in three females aged more than 6 months, which did not show any signs of diarrhoea at the time of sampling. *Cryptosporidium* prevalence was not affected by sex in all studied animal species. The results of the present study provide the first data on the prevalence of *Cryptosporidium* spp. in dromedary camels and broiler chickens from steppe and high plateau regions in Algeria.

INTRODUCTION

Genus *Cryptosporidium* are parasites belong to the phylum Apicomplexa infecting mainly the gastrointestinal tract and occasionally the respiratory system of different vertebrates (Ryan, 2010; Xiao, 2010). To date, based on morphological, biological and molecular data, thirty-four species of *Cryptosporidium* have been described, including twenty-three in mammals, four in birds, three in fish, three in reptiles and one in amphibian (Ryan & Xiao, 2014; Ryan *et al*., 2014; Kvác *et al*., 2016; Holubovà *et al*., 2016; Jež ková *et al*., 2016; Zahedi *et al*., 2017). In addition, more than 50 genotypes have been identified in the different animal hosts from several countries (Fayer, 2010; Kvác *et al*., 2013). More than seventeen species have been detected in human, where *C. parvum*, C. *hominis* and *C. meleagridis* represent the most common species involved in the waterborne outbreaks in high-risk individuals such as the very young, the elderly and immunodeficient persons (AIDS patients) (Cacciò & Putignani, 2014; Zahedi *et al*., 2016).

Cryptosporidiosis associated mainly with *C. parvum* species is a major cause of diarrhoea in young farm animals such as calves, lambs, goat kids, piglets, and with lower level in foals (Santín & Trout,

2008; Perrucci *et al.*, 2011; Veronesi *et al.*, 2010; Laatamna *et al.*, 2015). In farmed animals, specifically in ruminants and poultry breeding (chickens and turkeys), cryptosporidiosis is responsible of clinical morbidity, mortality and associated with production losses (Robertson *et al.*, 2014).

In Africa continent, *Cryptosporidium* have been reported in several domesticated animal species, including cattle, sheep, goats, farmed buffalo, horse, birds (chickens and turkeys), pig, cultured tilapia (fish) and dog. However, most of the investigations have been focused on bovine cryptosporidiosis (Squire & Ryan, 2017). In Algeria, a little of studies carried out on animal cryptosporidiosis have been published. Cryptosporidiosis was reported in young calves and cattle (Baroudi *et al.*, 2017; Benhouda *et al.*, 2017; Hocine *et al.*, 2016; Khelef *et al.*, 2007; Ouchene *et al.*, 2012, 2014), broiler chickens and turkeys (Baroudi *et al.*, 2013), and in equines (horse and donkey) (Laatamna *et al.*, 2013; Laatamna *et al.*, 2015). To date, no published data are available on human cryptosporidiosis in Algeria (Squire & Ryan, 2017). The purpose of this study was to investigate the prevalence of *Cryptosporidium* spp. and associated potential risk factors in farmed animals, including cattle, sheep, broiler chickens and dromedary camels from different steppe and high plateau regions in Algeria.

MATERIALS AND METHODS

Study area and sampling

The study was performed in 69 livestock farms, which keep cattle, sheep, broiler chickens and dromedary camels, located in steppe and high plateau regions from five provinces in Algeria (Fig. 1). The farms were selected without previous knowledge of parasitological status. Faeces of 137 and 152 cattle were obtained from 10 and 14 farms in the rural areas of Tiaret and Ras El-Oued. The farms of Ras El-Oued area are small private breeding and they practice traditional nursing of the neonatal calves, which are left in contact with the cows. The same breeding system is adopted in the farms of Tiaret region, except two dairy farms characterized by acceptable hygiene conditions and adopting separation of calves from the cows.

Faeces of Sheep were taken from three provinces, including 125 samples collected from 18 private farms of steppe region of Djelfa, 75 samples obtained from five farms in Ras El-Oued area and 54 samples from four farms in Mila region. The sheep of the steppe region graze during almost all seasons on the pastures of allies, while animals of the high plateau areas (Ras El-Oued and Mila) pasture mainly in summer and spring seasons and kept during the cold and rainy season.

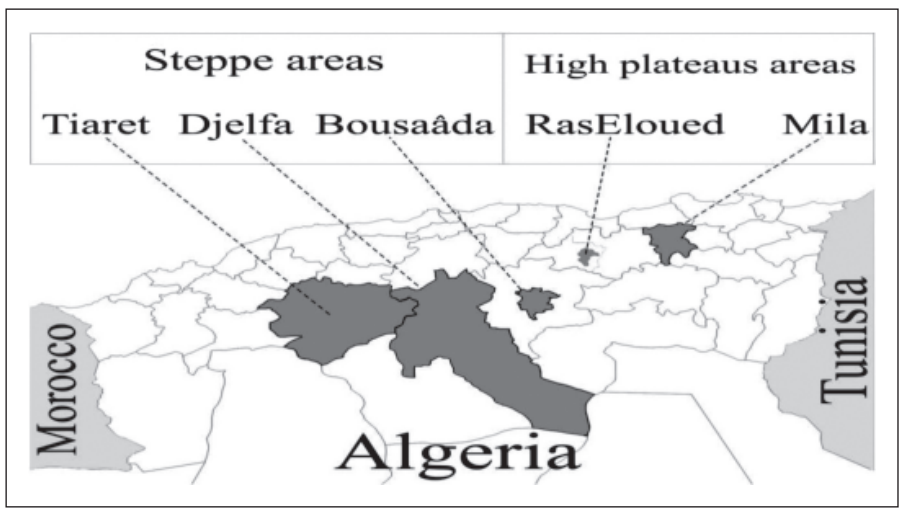

Figure 1. Geographic distribution of the study areas.

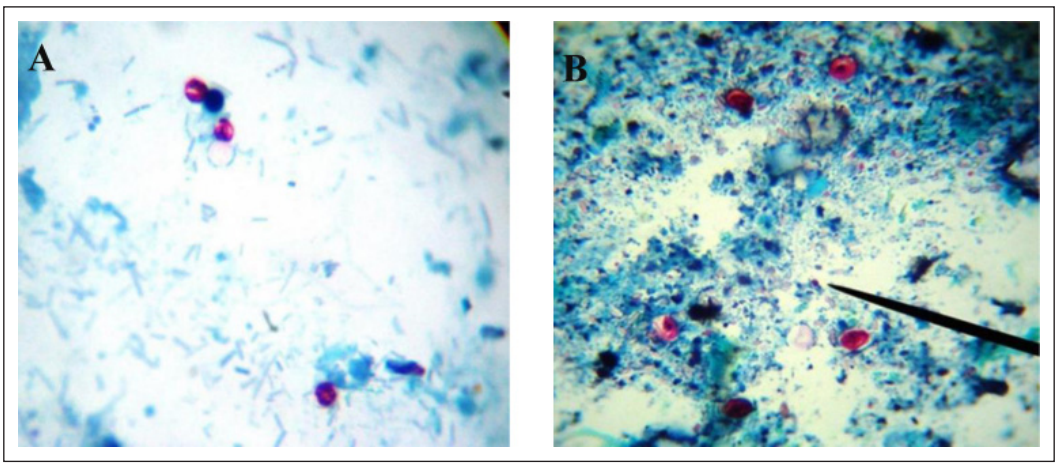

Figure 2. *Cryptosporidium* oocysts observed by modified Ziehl-Neelsen staining (Objective x100), (A) from neonatal calf and (B) from cow.

Faecal samples of 149 dromedary camels were obtained from eight farms, original of four localities situated in the steppe regions of Djelfa and Bousaâda. Animals are on pasture during the most time of year and some herds are under transhumance to get for more abundant pastures. Dromedary camels are used for milk and wool production.

Trachea samples of 135 broiler chickens were collected from 10 farms in the region of Djelfa. Broiler chickens are reared on soil in intensive livestock buildings for a period ranging between 55 to 60 days.

Sample collection

Faecal samples of cattle, sheep and dromedary camels were collected directly from the rectum or from the ground immediately after defecation. In broiler chickens, trachea tract was taken up from dead chickens after necropsy. Each sample was individually placed into a sterile plastic container and transported in an isotherm box to the laboratory. Potassium Dichromate (2.5%) was used for the conservation of faecal samples examined more than three days after the sampling. Sample collection was carried out just one time for each farm.

Coprological examination

All specimens were screened for the presence of *Cryptosporidium* oocysts by formalin-ether concentration method and modified Ziehl-Neelsen staining (Henriksen & Pohlenz, 1981). Obtained sediment of each faecal sample from formalin-ether concentration was used for preparing of smears which are stained with modified Ziehl-Neelsen acid-fast method and examined microscopically under oil immersion. The liquid of trachea of broiler chickens was examined using the same staining technique. A semi-quantitative method was used to estimate the degree of infestation in infected animals, basing on the calculation of oocysts number observed on the microscopic field, over whole of examined slide (objective x40). Nevertheless, this method cannot be considered as quantitative estimation because the number of oocysts varies considerably during the infection. A score for each positive sample was established as follows (Table 3): Low score (+):1 to 4 oocysts over the entire examined slide; Mean score (++): 1 to 10 oocysts per microscopic observed field; High score (+++): more than 10 oocysts per microscopic observed field (O.I.E., 2008).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS version 22.0) was used to perform the statistical analysis. The Chi-square test and Fisher exact were exploited to assess relationships between *Crypto-*

sporidium detection and animal attributes (age, sex, and presence or absence of diarrhoea). The confidence interval was fixed at 95% and the Chi-square and Fisher exact values were calculated with *P*-value of < 0.05 that was regarded statistically significant.

RESULTS

The prevalence of *Cryptosporidium* spp. in screened animal species in the different study areas is described in the Table 1.

In cattle, 61 out of 152 (40.1%) and 45 out of 137(32.8%) of examined samples were revealed positive for *Cryptosporidium* spp. respectively in Ras El-Oued and Tiaret areas. The prevalence of *Cryptosporidium* spp. varied significantly (p-value = 0.007) with age, whose the highest rate (52.6%) was observed in neonatal calves (< one month). There were no difference in *Cryptosporidium* detection between male and female (p-value = 0.88). Out of 23 diarrheic cattle, 14 animals showed *Cryptosporidium* excretion (11 neonatal calves, 2 calves aged of 1-3 months and one >6 months). In contrast, 92 (34.6%) among 266 animals with normal faeces were positive for *Cryptosporidium*. The presence of *Cryptosporidium* spp. was significantly associated with diarrhoea (p-value =0.012), particularly, 61.1% (11/18) of diarrheic neonatal calves showed oocysts shedding compared to non-diarrheic neonatal calves (30/60) (data not shown in the Table 2). From 22 traditional private farms characterized by bad hygiene conditions and close contact of calves with the cows, *Cryptosporidium* spp. was found in 38.2% (97/254) of screened animals, while 25.7% (9/35) of examined cattle from two dairy farms showed *Cryptosporidium* excretion. It seems that *Cryptosporidium* detection in traditional farms reached a high proportion compared to that observed in dairy farms, but statistically, no significant difference was noted between the positive cases recorded in these two different breeding systems (p-value = 0.151) (data not shown in the Table 2).

In sheep, *Cryptosporidium* was found in more of 80% of sampled farms, with respectively a prevalence of 22.7% (17/75), 22.2% (12/54) and 7.2% (9/125) in Ras El-Oued, Mila and Djelfa regions. The prevalence of *Cryptosporidium* spp. varied significantly with age of sheep (p-value = 0.007), in which the lambs aged between 1-6 months, followed by neonatal lambs were the most infected. *Cryptosporidium* presence did not vary significantly with sex (p-value = 0.515), and no positive sheep showed a diarrhoea at the time of sampling.

In broiler chickens, *Cryptosporidium* spp. was detected in 5 of 10 sampled farms, with an overall prevalence of 8.9% (12/135). Detection of tracheal infection was higher (9/30 = 30%) in broiler chickens aged of

Table 1. Prevalence of *Cryptosporidium spp*. in farmed animals from steppe and high plateau regions in Algeria

Animal species	Province	Number of sampled farms	Number of examined samples	Number of positive samples (%)
Cattle	Tiaret	10	137	45 (32.8)
	Ras El-Oued	14	152	61 (40.1)
	Subtotal	24	289	106 (36.7)
Sheep	Ras El-Oued	5	75	17(22.7)
	Mila	4	54	12(22.2)
	Djelfa	18	125	9 (7.2)
	Subtotal	27	254	38 (15)
Broiler chickens	Djelfa	10	135	12 (8.9)
Dromedary	Djelfa	5	90	02(2.2)
	Bousaâda	3	59	01(1.7)
	Subtotal	8	149	03 (2)

Table 2. Distribution of *Cryptosporidium* spp. by age, sex and presence or absence of diarrhea

Factors / Animal species		N° of positive animals / N° of examined animals (%)		
		Cattle	Sheep	Dromedary
Age	0-1 month	41/78 (52.6)	3/16 (18.7)	0/0 (00)
	1-3 months	18/52 (34.6)	30 /148 (20.3)*	0/49 (00)*
	3-6 months	07/25 (28)		
	> 6 months	40/134 (29.8)	5/90 (5.5)	3/100 (3)
Sex	Female	68/187 (36.4)	22/159 (13.8)	3/144 (2.1)
	Male	38/102 (37.2)	16/95 (16.8)	0/5 (00)
Diarrhea	Presence	14/23 (60.9)	0/0 (00)	0/0 (00)
	Absence	92/266 (34.6)	38/254 (15)	3/149 (2)

*Only sheep and dromedary camels aged of 1-6 months were included.

Table 3. Degree of infestation by *Cryptosporidium* oocysts in the different animal species

Degree of infestation (score)	Number of positive animals according each score			
	Cattle	Sheep	Dromedary	Broiler chickens
1–4 oocysts (+) (low score)	–	–	3	–
1–10 oocysts (++) (mean score)	88	38	–	12
> 10 oocysts (+++) (high score)	18	–	–	–
Total	106	38	3	12

16-24 days compared to birds aged of 35-44 days (3/85 = 3.5%). None of broiler chickens more than 44 days was found to be positive for *Cryptosporidium* (Data not shown in Table 2).

In dromedary camels, 25% (2/8) of sampled farms were found to be positive for *Cryptosporidium*, with an overall prevalence of 2% (3/149). Positive camels are females, aged more than 6 months and did not show any signs of diarrhoea at the time of sampling. Presence of *Cryptosporidium* spp. in dromedary showed no sex, age and diarrheal status association (P > 0.05 in all results).

Infected camels showed low score with 1-4 oocysts over the entire of examined slide. All positive sheep and broiler chickens showed a mean score with 1-10 oocysts per microscopic observed field. While, 17% of infected cattle (17 neonatal calves and one cow) indicated high score (> 10 oocysts), the rest (83%) including 24 neonatal calves, 18 calves aged of 1-3 months, 7 calves aged of 3-6 months and 39 cattle more than 6 months showed a mean score (data not shown in Table 3).

DISCUSSION

Bovine cryptosporidiosis has been reported worldwide (Robertson *et al.*, 2014; Santín & Trout, 2008). In Algeria, the overall prevalence (36.7%) of the present study is in agreement with the molecular report of Benhouda *et al.* (2017), in which *Cryptosporidium* was detected in 36.4% of examined cattle. However, previous studies in the centre and east of the country have indicated low prevalence rates of 13.7%, 17%, 22.6% and 22% (Baroudi *et al.*, 2017; Khelef *et al.*, 2007, 2002; Ouchene *et al.*, 2012). In contrast, Ouchene *et al.* (2014) reported a higher prevalence (50.8%) in three areas (Bejaia, Setif, and Souk Ahras) in North-eastern of Algeria. Similar situation in turkey, a prevalence of 64.3% was demonstrated

by Değ erli *et al.* (2005). Generally, in cattle, the prevalence often exceeds 50-60%, and the cumulative prevalence approaches 100% (Santín *et al.*, 2008).

Similar to the present report and previous studies conducted in Algeria and other countries, cryptosporidiosis is most common in younger calves (4 to 30 days) (Olson *et al.*, 1997; Quílez *et al.*, 1996; de la Fuente *et al.*, 1999; Robertson *et al.*, 2014). The increased sensitivity and the specific susceptibility to *Cryptosporidium* during this critical period should be explained by the characteristic status of the immune system of the neonatal calf. Any variability in presence of *Cryptosporidium* spp. between sex categories was not observed in the present and previous studies (Akam *et al.*, 2007; Hocine *et al.*, 2016; Dărăbus *et al.*, 2001; Paul *et al.*, 2008; Maurya *et al.*, 2013). On the other hand, a significant higher prevalence was recorded between sexes, where females were more infected than males (Bhat *et al.*, 2012, 2013; Mallinath *et al.*, 2009). Bovine cryptosporidiosis is considered worldwide a major cause of diarrhoea in young calves (up to 6 weeks of age) (Robertson *et al.*, 2014). Non-diarrheic animals (calves and adult cattle) showed a considerable infestation rate, so they play an important role in the environmental contamination. It seems that there is a relationship between the frequency of occurrence of *Cryptosporidium* cases in cattle and the hygienic measures adopted in farms, which is shown in the present study and reported previously in some studies (Khelef *et al.*, 2007; Samie *et al.*, 2017). In addition, *Cryptosporidium* infection in calves from dairy and beef herds remains housing-dependent (Kváč *et al.*, 2006).

Ovine Cryptosporidiosis can be found throughout the world. The prevalence varies widely between studies from 0% to 77% (Robertson *et al.*, 2014). Currently, only Dahmani *et al.* (2017) have reported the first data on sheep cryptosporidiosis in steppe region of Aïn Oussera (Algeria) with comparable prevalence (14.6%). In Africa, ovine cryptosporidiosis was reported respectively in Egypt, Tanzania, Tunisia and Zambia with a prevalence of 2.5%, 22.2%, 11.2% and 12.5% (Mahfouz *et al.*, 2014; Parsons *et al.*, 2015; Soltane *et al.*, 2007; Goma *et al.*, 2007). In sheep from Spain, Norway and Australia, *Cryptosporidium* was detected in 59% of examined animals and 84% of the farms surveyed tested positive, 15% and 24.5% respectively (Causapé *et al.*, 2002; Robertson *et al.*, 2010; Yang *et al.*, 2009). The present study indicated a higher occurrence of *Cryptosporidium* spp. in lambs aged of 1-6 months and neonatal lambs (< 1 month) than in sheep aged more of 6 months. Similar results were obtained in sheep from Egypt, Tunisia, Brazil and Maryland (Mahfouz *et al.*, 2014; Soltane *et al.*, 2007; Fiuza *et al.*, 2011; Santín *et al.*, 2007). In India, neonatal lambs were significantly more infected (65%) than other two age groups (1-3 and 3-6 months) (Ahamed *et al.*, 2013). In Spain, presence of *Cryptosporidium* was also more frequently in lambs aged between 1-21 days (66.4%) than in those aged between 22 and 90 days (23%) (Causapé *et al.*, 2002). The prevalence rate may decrease with advancing age and the development of the immune system. In contrary to the present study, female lambs (51.6%) were found more infected than males (39.3%) (Ahamed *et al.*, 2013). It seems that the sex of sheep is not known as an associated risk factor. Excretion of *Cryptosporidium* spp. in sheep mainly in lambs from Algeria, Tunisia, Egypt and Zambia was not associated with signs of diarrhoea. In contrast, it has been stated that this protozoan (*C. parvum* species) is of the most important pathogens associated with diarrhoeal disease and mortality in neonatal lambs (Causapé *et al.*, 2002; de Graaf *et al.*, 1999; Quílez *et al.*, 2008). Ahamed *et al.* (2013) have reported that diarrheic lambs showed significantly higher prevalence (54.4%) than non-diarrheic lambs (34.6%). Infection of sheep by some species of *Cryptosporidium* not associated with diarrhoeal disease (example: *C. bovis*) may explain probably absence of diarrhoea in infected animals. All age groups of infected sheep of the present study had a mean score of infection (1-10 oocysts per microscopic observed field). In Turkey, Sari *et al.* (2009) have found 58.1% (90/155), 18.7% (29/155) and 23.2% (36/155) of infected lambs had respectively a mild, average and severe

infection. The extent of excretion of *Cryptosporidium* oocysts by infected animals could vary during the course of infection and this shedding variation may be depending on several factors (age and clinical status).

A few studies on cryptosporidiosis of broiler chickens were documented in Africa (Algeria, Tunisia, Morocco and Ivory Coast) (Squire & Ryan, 2017), where most of them reported a prevalence varied of 4.5% to 34.4% in the faecal samples of examined birds (Baroudi *et al.*, 2013; Soltane *et al.*, 2007; Kichou *et al.*, 1996; Berrilli *et al.*, 2012). In the present study, *Cryptosporidium* was detected in the trachea of 8.9% (12/135) of examined broiler chickens, from 50% of sampled farms. In Morocco, tracheal infection by cryptosporidia was identified in 37% (14/38) of investigated farms with an overall prevalence of 2% (5/225) (Kichou *et al.*, 1996). A higher prevalence of respiratory infection (tracheal tract) has been demonstrated in USA (10-60% in 41% of collected farms) and Japan (24%) (Goodwin *et al.*, 1996; Itakura *et al.*, 1984).Contrary to the present study where the detection of parasite is higher (9/30=30%) in broiler chickens aged of 16-24 days than in those aged of 35-44 days (3/85=3.5%), in Morocco, the highest occurrence of *Cryptosporidium* spp. (52% and 43%) was found respectively in 36-45 and >45 days-old chickens (Kichou *et al.*, 1996).The infection of the respiratory tract by *Cryptosporidium* spp. has been reported to cause disease in chickens (mortality, weight loss, tracheitis, airsacculitis and condemnation of birds), but subclinical cryptosporidiosis has been also observed frequently (Fernandez *et al.*, 1990; Goodwin *et al.*, 1996; Robertson *et al.*, 2014). This study reports, for the first time, the presence of cryptosporidia in the trachea of broiler chickens in Algeria. So, more thorough investigations are needed to better understand avian respiratory cryptosporidiosis and fully assess its economic impact in poultry.

The prevalence of cryptosporidiosis in dromedary camels has not been yet documented in Algeria, which is low in the present study (2%) compared to results of different previous reports conducted in Egypt (19.3%, 17.5%), Iraq (61%) and different areas of Iran (37.9%, 20.3%, 16.9% and 10%) (Abdel-Wahab & Abdel-Maogood, 2011; El-Kelesh *et al.*, 2009; Hussin *et al.*, 2015; Razawi *et al.*, 2009; Sazmand *et al.*, 2012; Nazifi *et al.*, 2010; Yakhchali & Moradi, 2012). This low prevalence is in agreement with studies of Saleh and Mahran (2007) and Borj *et al.* (2009) carried out also in Egypt and Iran that showed an infection rate of 3.4% and 1.9% respectively. While, no positive cases (0%) were identified in examined camels from Tunisia, Iraq and Portugal (Soltane *et al.*, 2007; Mahdi & Ali, 1992; Alves *et al.*, 2005). No variability in presence of *Cryptosporidium* spp. by age groups was observed in the present report and those conducted in Iran (Razawi *et al.*, 2009; Sazmand *et al.*, 2012). In contrast, other findings have indicated that the age of examined camels had a significant effect on the prevalence (Yakhchali & Moradi, 2012; Saleh &Mahran, 2007; Hussin *et al.*, 2015). Sex was not reported as associated risk factor in prevalence variation of *Cryptosporidium*. In camels, few data are available on association of infection with diarrheal disease. No signs of diarrhoea were observed in positive dromedary camels in the present study. Yakchali and Moradi (2012) have reported significantly a higher prevalence in camel calves (<1-year-old) (20%) than other age groups, in which the diarrheic camel calves had the highest prevalence (16%), in comparison to that in non-diarrheic adults (6.5%). Future studies should be carried out to better understand the epidemiology (prevalence) and the involvement of this protozoan in the clinical cryptosporidiosis.

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Conflict of interests

The authors declare that they have no conflict of interest.

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