Prevalence of *Cryptosporidium* spp. in ruminants of Lorestan province, Iran

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Abstract Cryptosporidiosis is one of the important zoonotic diseases caused by an intracellular protozoan parasite called *Cryptosporidium*. This study aimed to investigate the prevalence of *Cryptosporidium* spp. infection on 1,115 ruminants, cattle, sheep and goats, in Lorestan province, Iran. Using formol–ether concentration technique and modified Ziehl–Neelsen staining method afterwards, the overall prevalence of *Cryptosporidium* spp. infection in ruminants of Lorestan province was 7.17 %. Prevalence of infection was 9.07 % (39 of 430), 5.80 % (20 of 345) and 6.18 % (21 of 340) for cattle, sheep and goats respectively. There was no significant difference between contamination of all examined animals and different geographical and climatic situations ($P > 0.05$) and diarrhea was not directly associated with *Cryptosporidium* infection ($P > 0.05$). In conclusion, the prevalence of cryptosporidiosis in Lorestan province was relatively low, but it should be noticed that this opportunistic parasite is zoonosis and also can make epidemics in ruminants as well as human population in suitable conditions.

Keywords *Cryptosporidium* spp. · Ruminants · Epidemiology · Iran

Introduction

Cryptosporidium spp. are intracellular and ubiquitous protozoan parasites belong to phylum Apicomplexa, infecting wide range of vertebrates (Xiao et al. 2004). This parasite has intracellular habitat, but its correct location is between brush borders of epithelial cells which always mentioned as extra-cytoplasmic organism (Fayer and Xiao 2008). *Cryptosporidium* has monoxenous life cycle and the principal route for transmission is fecal-oral by environmentally resistance oocysts (O'Donoghue 1995). Thus far, multitude waterborne outbreaks have been made difficulties in human crowds especially for immunocompromised patients and also produced considerable economic losses in livestock (Fayer and Xiao 2008). Cryptosporidiosis is relatively an emerging disease and recognized usually as one of the principal causes of diarrheal diseases in human and livestock (Santín 2013; Fayer et al. 2000).

Cryptosporidium parvum, *Corynebacterium bovis*, *Cryptosporidium andersoni* and *Cryptosporidium ryanae* are the four common Cryptosporidia that infect ruminants, but *C. parvum* is a dominant species and in the meantime *C. parvum* has a great zoonotic importance (Ryan et al. 2003; Wang et al. 2008; Santín 2013; Fayer and Xiao 2008). In ruminants, the clinical principal manifest of cryptosporidiosis is diarrhea that occur chiefly in young animals. The disease may lead to dead in neonates especially when combine with other intestinal infections such as viral diarrhea (Fayer and Xiao 2008).

Although, the prevalence of *Cryptosporidium* infection has studied in many areas of Iran, but there is no available

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documented data on this issue in ruminants of Lorestan province. Thus, the main aim of this study was to investigate the prevalence of *Cryptosporidium* spp. infection in normal and diarrheic ruminants of Lorestan province, south-western, Iran which is an important area of livestock industry (Fig. 1).

There are many diagnostic tools for identification of Cryptosporidia in stool samples including various staining procedures as well as molecular techniques, but for large scale studies molecular tools are not the methods of choice because they are very expensive and also time consuming. In this regards, such techniques are very helpful for genotyping purposes to identify the precise species or subspecies of *Cryptosporidium* (Bull et al. 1998; De Quadros et al. 2006; Thompson et al. 2007; Fayer and Xiao 2008). So, we decided to perform acid-fast modified Ziehl–

Neelsen staining for primary prevalence investigation of this protozoa in the area of study.

Materials and methods

This cross-sectional study was undertaken between November 2013 and August 2014 in different geographic locations with three different weather conditions cold (north area), hot (south area) and temperate (central area) climates of Lorestan province in Iran. This area is one of the populous districts and has an important situation in livestock industry in Iran. Cluster random sampling was done for fresh stool sample gathering directly from the rectums of 1,115 non-diarrheic ($n = 979$) and diarrheic ($n = 136$) animals including 430 cattle (adult = 335, calf = 95) 345 sheep (adult = 250,

Fig. 1 Map of lorestan on Iran

Table 1 Prevalence of *Cryptosporidium* infection in diarrheic and non-diarrheic young and adult ruminants of Lorestan province, Iran

Animals	Cattle (n = 430)		Sheep		Goat	
	Adult (n = 335)	Calf (n = 95)	Adult (n = 250)	Lamb (n = 95)	Adult (n = 225)	Kid (n = 115)
C+*, non diarrheal	32 (9.55 %)	4 (4.2 %)	15 (6 %)	2 (2.1 %)	17 (7.55 %)	2 (1.74 %)
C+, diarrheal	0 (0 %)	3 (3.16 %)	0 (0 %)	3 (3.16 %)	0 (0 %)	2 (1.74 %)
Total	32 (9.55 %)	7 (7.37 %)	15 (6 %)	5 (5.26 %)	17 (7.55 %)	4 (3.48 %)

* Positive for *Cryptosporidium*

lamb = 95) and 340 (adult = 225, kid = 115) goats of different ages. The specimens have been transported quickly in sterile plastic bottles to the laboratory and oocysts were purified using the formol–ether concentration technique and the sediment was examined for the existence of *Cryptosporidium* oocyst by modified Ziehl–Neelsen staining procedure afterwards (Fayer and Xiao 2008).

Briefly, thin stool smears were prepared on microscopic slides and dried in ambient temperature. Smears were fixed using absolute methanol and stained by basic fuchsin solution for 15 min and non-*Cryptosporidium* materials decolorized by acid–alcohol solution for 15 s afterward. Then, the slides were soaked in a jar containing malachite green solution for 20 s, washed, dried and examined for presence of the oocysts under light microscopy. *Cryptosporidium* oocysts were identified according to morphological criteria (Lindsay et al. 2000; Fayer and Xiao 2008).

The data were analyzed statistically by Chi square statistical test (χ^2 test) and fisher's exact test using SPSS software (Version 18). Alpha was 0.05 for all the tests and 95 % confidence intervals were calculated for the association between the prevalence of *Cryptosporidium* in animals with age, stool condition and geographical distribution.

Results

The oocysts of *Cryptosporidia* were detected in 80 of 1,115 (7.17 %) examined animals. The overall prevalence of infection was 9.07 % (39 of 430), 5.80 % (20 of 345) and 6.18 % (21 of 340) for cattle, sheep and goats respectively. Details on prevalence of infection in young and adult animals are presented in Table 1.

There were no significant difference between contamination of all examined animals and different geographical and climate situations ($P > 0.05$). Furthermore, diarrhea was not directly associated with *Cryptosporidium* infection ($P > 0.05$).

Discussion

A zoonotic parasite, *Cryptosporidium*, affects a wide range of livestock and human, resulting complications, especially

due to serious economic loss and public health concern. *Cryptosporidiosis* causes enteric disorder and is a common cause of diarrhea in neonatal calves (Fayer and Xiao 2008; Hamnes et al. 2006; Geurden et al. 2007; Thompson et al. 2007). The disease is one of the important health problems in both developed and undeveloped countries with increasing concern in immunocompromised patients (DuPont et al. 1995; Kjos et al. 2005; Harp 2003).

At yet, several tools have been studied for detection of the oocysts of *Cryptosporidium* including some microscopic methods and molecular diagnostics as well. Molecular techniques are expensive, time consuming and laborious methods for distribution studies or screening, however such tools are methods of choice when we aim to detect genotypes of *Cryptosporidium* species. For innate and large scale studies, microscopy is more successful method when combined with concentration techniques and staining of fecal smears with appropriate stain and well modification of the procedure since there are many particles in fecal specimens that may confuse the microscopists. With this regards, modified Ziehl–Neelsen method is mentioned as a reasonably useful tool to distinguish the *Cryptosporidium* (Bull et al. 1998; De Quadros et al. 2006; Thompson et al. 2007; Fayer and Xiao 2008).

At our knowledge, the current investigation is the first documented study on *Cryptosporidium* in ruminants of Lorestan province in Iran. Our results demonstrated relatively low prevalence (7.2 %) of *Cryptosporidium* infection in ruminants of Lorestan province and this prevalence was not statistically different in cattle, sheep and goats by modified Ziehl–Neelsen staining. This finding is somewhat in conformity to reports of Romero-Salas et al. (2012), Santín (2013) and Azami (2007). Furthermore, we did not see significant higher infection in diarrheic animals in comparison with non-diarrheic animals. As, several reports revealed correlation between higher infection rate with *Cryptosporidium* in cattle and calves, we deduced that in the area of our study diarrhea may not directly related to cryptosporidiosis (Uga et al. 2000; Nasir et al. 2009; Asadpour et al. 2013; Silva et al. 2013; Mirzai et al. 2014). On the other point of view, presence of the parasite in livestock of Lorestan province affirms the public health importance, especially in people that are directly related to

the animals. These infected animals are also potential sources of infection for other animals or public crowds to make *Cryptosporidium* epidemic in appropriate situations. *Cryptosporidium* epidemics have been frequently happened in recent years (Guyot et al. 2001).

According to statistical analyses significant correlations were not determined between infection rates and age. We did not consider different seasons in our study, but our findings reveals no statistically difference for *Cryptosporidium* infection rate in various weather conditions of Lorestan province. Becher et al. (2004) showed that season had no effect on prevalence of the infection. There are many different deductions represented by other works on effects of season on prevalence of cryptosporidiosis. For instance, some studies showed that the prevalence of cryptosporidiosis in calves and cattle was higher in winter (Garber et al. 1994; Mohammed et al. 1999). According to Bull et al. (1998) and Torres et al. (2000) reports in wild animals, the prevalence of cryptosporidiosis was not age depended. On the other hands, in Iran, some studies found significant relationship among *Cryptosporidium* infection and age (Gharekhani et al. 2014; Heidarnegadi et al. 2012).

In conclusion, according to our results we deducted that the prevalence of cryptosporidiosis in Lorestan province is relatively low in comparison with other parts of Iran, but it should be noticed that this opportunistic parasite is zoonosis and also can make epidemic in ruminants as well as human population in suitable conditions.

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Conflict of interest The authors declare that there is no conflict of interest.

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