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Prevalence of Cryptosporidium Infection among Livestock and Humans in Contact with Livestock in Hamadan District, Iran, 2012

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ABSTRACT

Background: *Cryptosporidium* is a protozoan parasite causes cryptosporidial diarrhea, which is typically a short-lasting benign infection, but can become severe and non-resolving in immunocompromised individuals. The aim of the present study was to determinate the prevalence of *Cryptosporidium* infection in livestock and humans that were in contact with livestock in Hamadan district, Iran.

Methods: In this cross sectional study a total of 660 fecal specimens were collected; 228, 195 and 237 from humans, whom raising livestock, their calves and lambs/goats, respectively in spring 2012. Samples were concentrated by formalin-ether concentration technique and examined using cold modified Ziehl-Neelsen staining method.

Results: Two (0.87%) out of 228, 25 (12.8%) out of 195 and 6 (2.5%) out of 237 fecal samples of humans, calves and lambs/goats were positive for *Cryptosporidium* oocysts, respectively. There was no significant relationship between the infection to *Cryptosporidium* and demographic variables of humans. However, *Cryptosporidium* infection rate was higher in diarrheic calves (OR=3.81; 95% CI: 1.30, 11.21; *P*=0.010).

Conclusion: Despite studies conducted in some regions of Iran that resulted in a relatively high rate of infection in humans in contact with livestock, our results showed low prevalence and low carrier status in the asymptomatic persons in Hamadan region. Because the infection in calves and lambs/goats was relatively high, these animals could be probable reservoir of infection for humans in this area.

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Introduction

Cryptosporidium is an apicomplexan intestinal protozoon, which infects animals and humans gastrointestinal tract and causes cryptosporidiosis[1,2]. The infection causes cryptosporidial diarrhea, which is commonly a short-lasting benign diarrhea. In addition it can cause severe diarrhea in immunocompromised individuals[2,3]. The parasite is transmitted by oocysts shed by hosts, mostly animal reservoirs such as cattle and sheep. The parasite has ability to infect a wide variety of host range[4]. Humans and cattle may be infected by the bovine genotype, *C. parvum*. Although *C. hominis*, which is human genotype commonly infects only man[5]. In addition, *C. felis*, *C. meleagridis* and *C. muris* can infect humans[2]. The infective dose is notably low and about 10 oocysts can infect human[6].

Epidemiologically, cryptosporidiosis is a worldwide infection and present in every region, but not in Antarctica and commonly infection is frequent in warm and wet seasons[2]. About 300,000 people in the United States is thought to be infected by *Cryptosporidium* yearly[7]. In comparison with giardiasis, the incidence of *Cryptosporidium* infection is approximately 40%[8]. Because of the similar oocysts morphology between *Cryptosporidium* species, the role of animals, especially livestock, in human cryptosporidiosis is not clear completely. Cattle have been thought to be a crucial zoonotic source of cryptosporidiosis of humans. Small outbreaks of the infection have been reported in humans in contact with infected calves, for instance; "veterinary students, research technicians and children attending agricultural camps"[9].

In Iran, like other countries, the epidemiological studies have been conducted in some parts of the country, some of which emphasized on the zoonotic aspect of the infection and reported a wide range of infection from 0% to 32% in different societies[10,11].

The aim of the present study was to determine the prevalence of *Cryptosporidium* infection in calves, lambs/goats and involved peoples to find out the relationship between the infection in livestock and humans in contact with them during spring 2012 in Hamadan district, west of Iran.

Methods

In this cross sectional study 228, 195 and 237 fecal samples were collected randomly from humans, whom were in direct and indirect contact with livestock, their suckling calves and lambs/goats, respectively in spring 2012. Animal samples were collected from livestock of 50 owner families from 7 villages and also 7 dairy farms in Hamadan district, west of Iran. Demographic variables of people were collected through interviewing which include their sex, age, occupation, educational status, having direct or indirect contact with livestock, which animal they were in contact with and gastrointestinal symptoms they might had, and livestock information collected by direct observation and questioning from owners which include being diarrheic and non diarrheic. Moreover all suckling animals were under 3 months old.

Fecal samples of animals were taken directly from their rectum. The fecal samples were fixed quickly possible in 10% formalin for preserving until the examination. After sampling was ended, the preserved samples concentrated by formalin-ether concentration technique and examined using cold modified Ziehl-Neelsen staining method for microscopy, according to the following procedure. Thin smears prepared with concentrated materials and non concentrated fecal samples. Air dried fecal smears fixed with methanol and then stained by carbol fuchsine for 15 min, decolorized by 1% hydrochloric acid in methanol for 30 s, then slides re-stained with counter stain in 0.4% malachite green for 30 s; rinsed in tap water for washing of excess stain and dried in air[12,13]. The *Cryptosporidium* oocysts observed as spherical red colored objects, around 4-5µm in diameter, with internal structures somehow crescent shape (Figure 1). Data were analyzed using SPSS v.16 Software and chi-square test.

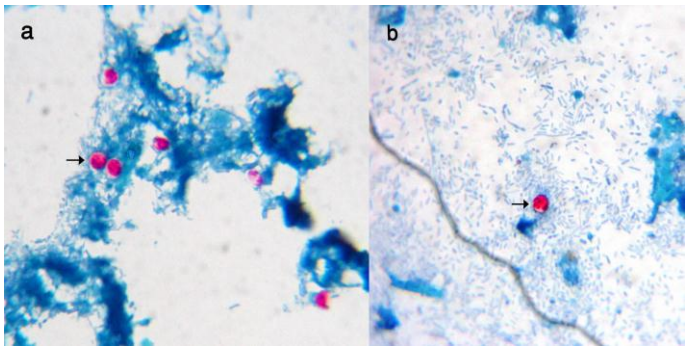

Figure 1: *Cryptosporidium* oocysts in calf (a) and human (b) feces stained with cold modified Ziehl-Neelsen staining method (Original)

Results

Totally 228 concentrated human fecal samples were examined and *Cryptosporidium* oocysts were observed only in 2 (0.87%) samples, but no oocysts were seen in non concentrated fecal smears. Twenty five (12.8%) out of 195 and six (2.5%) out of 237 fecal samples of calves and lambs/goats were positive equally in direct and concentrated smears, respectively. There was no statistically relationship between the infection rate and people mentioned demographic variables and the kind of animal they were in contact with.

Beside all, *Cryptosporidium* infection rate was significantly higher in diarrheic calves comparing to non diarrheic ones (OR=3.81; 95% CI: 1.30, 11.21; *P*=0.010). There was no significant difference regarding infection rate between diarrheic and non diarrheic lambs/goats (OR=1.51; 95% CI: 0.17, 13.42; *P*=0.709). In the studied livestock, distribution of the infection was relatively equal in both sexes. No correlation between the infection rate in people in direct and indirect contact with livestock has been observed (OR=0.44; 95% CI: 0.03, 7.13; *P*=0.552) (Table 1). No difference has been seen as for infection rate of animals in concentrated samples comparing to non concentrated samples, but in human asymptomatic cases no oocysts observed in non concentrated fecal samples.

Table 1: Odds ratio (OR) estimate of cryptosporidium infection among calve and lambs/goat with and without diarrhea and subjects having direct and indirect contact with livestock

Subjects	infection		OR	95% CI	*P* Value
	Absent	Present			
Calve					
Non diarrheic	157	19	1.00		
Diarrheic	13	6	3.81	1.30, 11.21	0.010
Lambs/goat					
Non diarrheic	204	5	1.00		
Diarrheic	27	1	1.51	0.17, 13.42	0.709
Humans in contact with livestock					
Indirect	69	1	1.00		
Direct	157	1	0.44	0.03, 7.13	0.552

Discussion

Totally, 660 fecal samples were collected from humans, suckling calves and lambs/goats. The overall prevalence was 5%. The infection of humans showed low rate in this area. The concentrating increased the possibility of detecting the infection in asymptomatic carriers. Infection rate in calves was 12.8%, which shows high infection rate. Accordingly, 2.5% of lambs/goats were infected. Results reveal that the *Cryptosporidium* infection is present in humans and livestock animals in Hamadan, however, a report from diarrheic children confirmed cryptosporidiosis in them about 5.6% [14].

Some similar studies have been carried out in different regions of the country that mostly reported higher prevalence in the similar study groups. For example, Sazmand et al. in 2012 conducted a study on camels and involved people and reported 32% of the studied people in contact with camels in winter as infected. Furthermore, the infection rate in camels was 22.6% that shows lower preva-

lence than humans. The prevalence they reported was even higher than HIV positive individuals' rates of 26.7%[15] and 9.1% [16] in two distinct studies in Iran. The infection is more prevalent and sever in immunocompromized individuals because of lack of effective immunity against organism[15,17], but the people in contact with the infected livestock are immunocompetent. The higher prevalence in the mentioned study shows the potential zoonotic aspect of the infection in people involved in farming activities, but our study does not support this idea in Hamadan area.

In our finding, the prevalence of the infection was 0.87% that illustrates somehow low prevalence comparing to the studies conducted in diarrheic patients and asymptomatic children rate of 2.5% to 4% of infection in Iran[10,18,19]. Ranjbar et al. in 2011 proposed the infected animals as a risk factor for *Cryptosporidium* infection in children in Babol, northern Iran. They found the infection rate in children was higher than in calves, 10.67% and 4%, respectively. Although they suggested that the infection rate in children from rural area was similar to urban areas[19]. Given to the fact that most of people in rural areas are farmers that are potentially in contact with infected livestock, their findings shows farm animals are not a serious risk for *Cryptosporidium* in children. In the other similar study done on the intestinal protozoan infections in rural inhabitants of Mazandaran Province, northern Iran, the authors found 0% *Cryptosporidium* infection in 855 fecal samples[20]. Nouri et al. investigated the prevalence of the *Cryptosporidium* oocysts in feces of non diarrheic shepherds and their non diarrheic sheep in Lorestan Province, Iran. They reported 17.2% and 13% infection rate in sheep and humans, respectively[21]. Azami et al. in 2007 reported the infection rate of 10.8% in calves in Isfahan, Iran[22]. In our study no significant relationship was observed between infection rate in the people who were in direct and indirect contact with livestock. This may be caused by immunity they acquired in constant contact with infective oocysts shed by farm animals.

Sampling and traveling to the rural area were major limitations in this study. Taking fecal samples from families was a real limit for our study, which was time and budget consuming. In addition, because the prevalence of *Cryptosporidium* infection of humans in Hamadan region is very low, greater sample size is needed to investigate the responsible source of human infection in Hamadan region.

Conclusion

Despite the fact that some authors reported a relatively high rate of infection in humans in contact with livestock in some part of the country, which shows the possible zoonotic aspect of the infection, our results showed low prevalence and low carrier status in the asymptomatic people in Hamadan region and also the zoonotic aspect of the infection has low effect on the epidemiology of infection in this region. In addition, according to our findings, concentration method increases the sensitivity of microscopic examination in asymptomatic human carriers, but not in symptomatic and asymptomatic animals. Because the infection rate of the studied animals was relatively high, especially in calves, these animals could be a probable reservoir of infection for humans in this area.

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Conflict of interest statement

The authors have no conflict of interest.

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