

Tehran University of Medical Sciences Publication http:// tums.ac.ir

# **Iranian J Parasitol**

Open access Journal at http://ijpa.tums.ac.ir



Iranian Society of Parasitology http://isp.tums.ac.ir

# **Original Article**

## Cryptosporidium Spp. Infection in Human and Domestic Animals

SM Heidarnegadi<sup>1</sup>, M Mohebali<sup>1, 2</sup>, Sh Maraghi<sup>3</sup>, Z Babaei<sup>4</sup>, Sh Farnia<sup>1</sup>, A Bairami<sup>1</sup>, \*M Rzaeian<sup>1, 2</sup>

 <sup>1</sup>Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran
 <sup>2</sup>Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran
 <sup>3</sup>Dept. of Medical Parasitology and Mycology, School of Medical, Jundishapur University of Medical Sciences, Ahwaz, Iran
 <sup>4</sup>Dept. of Medical Parasitology and Mycology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

#### (Received 14 Jun 2011; accepted 19 Dec 2011)

## ABSTRACT

**Background:** *Cryptosporidium* spp. is a coccidian parasite infected humans and animals. Prevalence rate of *Cryptosporidium* spp. infection associated with is some parameters such as sampling, age, season, country and contact to domestic animals. This study aimed to determine *Cryptosporidium* spp. Infection in humans and some animals in rural areas of Shushtar district from Khuzestan Province, south- west of Iran.

**Methods**: In this study, Stool specimens were randomly collected from 45 cattle, 8 buffalos, 35 calves, 22 turkeys, 3 sheep, 2 geese as well as 62 humans in different seasons selected from rural areas of Shushtar district located in Khuzestan in the south- west of Iran from August 2009 to April 2011. The collected stool samples were examined by modified Ziehl-Neelsen staining method.

**Results:** Altogether, 68/115 (59.1%) domestic animals and 9/62 (14.5%) of humans were showed *Cryptosporidium* spp. infection in the study areas.

**Conclusion**: In this study we found the high frequency of *Cryptosporidium* spp. infection in the studied areas.

Keyword: Cryptosporidium infection, Human, Domestic Animal, Iran

\*Corresponding author: Tel: +98 21 88951392, E-mail: rezaiian@sina.tums.ac.ir.

# Introduction

ryptosporidium is a protozoan parasite that infects the gastrointestinal tract of a wide rang of vertebrates including humans, livestocks, wild animals and birds (1). Cause by covering the extensive host rang, Cryptosporidium has been considered to be a zoonotic protozoa (2, 3). Cryptosporidium infection can persist for a long time and can lead to serious complications in patients with AIDS (4). But, in patients with an immune system, this organism leads to a self limited infection. Epidemiological studies have publicized that the most important ways of transmission are water born, human-animal and person to person contact (5). Cryptosporidium spp. is a main pathogen causing acute diarrhea, nonspecific signs including for instance dehydration, fever, anorexia, and weakness may be accompanied. Diarrhea is typically selflimiting in immunocompetent humans. However, it can be major public health importance in children as well as in immunocompromized individuals (6).

This study was designed to investigate the prevalence of *Cryptosporidium* infection in cattle, buffalo, turkey and human especially children exposed with livestock in tropical region of Khuzestan southwestern Iran.

### **Materials and Methods**

#### Fecal sampling

Stool specimens were collected during the different seasons from 45 cattle, 8 buffalo, 53 calves, 22 turkeys, 3 sheep, 2 geese and 62 humans randomly from rural areas of Shushtar City for example Chamtarkhan, Konaarpir, Gelalak and Moraz village in Khuzestan Province, south- west of Iran, between August 2009 and April 2011 (Table 1). For animals, a single fecal sample was collected from the rectum of each animal

using disposable plastic bag and transferred to a wide-mouthed disposable plastic container. The specimens were transported to the Intestinal Protozoa Laboratory, School of Public Health, Tehran University of Medical Sciences and preserved in potassium dichromate 5% at 4°C until examined.

#### Cryptosporidium oocyst detection

Fecal specimens were concentrated by both formol-ether concentration and sheater's flotation (7, 8). Seven ml of the formalintreated stool specimen and 3 ml of ethyl ether was centrifuged at 650 g for 2 min, resulting in four layers: a layer of ethyl ether, a plug of debris, a layer of formalin-saline, and the sediment. The plug was removed with an applicator stick and the supernatant three layers were decanted. One drop of the sediment poured on to slide and prepared on two microscopically smears were prepared from the sediment and stained by the acidfast staining. Both of cold and hot method of Modified Ziehl-Neelsen staining was used (9).

#### Statistical analysis

Data were analyzed using SPSS (version 13.5; SPSS, Inc, Chicago, IL, USA). A Chisquared test was used to compare the differences in prevalence of *Cryptosporidium* spp. oocysts between samples of livestock and human with season, age, sex and clinical sign at a 5% level of significance. The prevalence rates were calculated with 95% confidence intervals.

### Results

#### Frequency of Cryptosporidium spp.

The overall frequency of *Cryptosporidium* spp. oocysts in animals was 59.1% (68/115) and in human 14.5% (9/62). The highest infection rate of *Cryptosporidium* spp. among

animals was 74.5 % (38/51) in winter and the lowest in summer 10% (1/10), the infection rate in spring and autumn were 57.5% (23/40), 42.8% (6/14) respectively. The prevalence result of *Cryptosporidium* spp. oocysts among the various animals in different seasons is presented in Fig. 1.

We have found a statistically significant relationship between infection of cryptosporidiosis and season (P < 0.05) in animals. The infection rate of *Cryptosporidium* spp. in animal at different sampling regions was 81% in Chamtarkhan, 40% in Konaarpir, and 60.9% in Gelalak and Moraz village. The prevalence results of *Cryptosporidium*  spp. among human for age and clinical sign are showed in Table 1.

We have not found a statistically significant relationship between infection of cryptosporidiosis and clinical signs and also human sex as well as age. Human contact with animals was 27.7% (10/36) and 12 % (3/26) in males and females respectively. Microscopic examination indicated that 40% (6/15) of humans were infected in spring, 7.6% (1/10) in summer, and 23.8% (5/21) in winter. The evaluation of the feces collected showed that infection of *Cryptosporidium* spp. in human in Chamtarkhan was 9.09% (1/11), Gelalak 42.3% (11/26), and Moaraz 4% (1/25).



Fig. 1: The distribution of *Cryptosporidium* spp. infected among the various animals in different seasons

Age (yr)	No. of posi- tive cases	Asymptomatic		Symptomatic	
		No.	%	No.	%
≤10	7	2	22.2	5	55.5
>10	2	1	11.1	1	11.1
Total	9	3	33.3	6	66.6

**Table 1:** Distribution of Cryptosporidium spp. infection in humans

## Discussion

The prevalence (59.1%) of Cryptosporidium spp. in animals obtained in this study was compared with other studies including 6.2% of cattle in Isfahan (10), 35.3% of turkey in the north and west provinces (11), 18.9% of cattle in Kerman (12), and 18.8% of cattle in Qazvin (13). This information indicated that the prevalence of Cryptosporidium in this area is higher than in other parts of Iran. Frequency of infection in cattle has been reported from different parts of the world with nearly 40% in Germany, 45.5% incidence in USA, 20% of calves in Canada, 19% of calves in Spain and 27% in Hungary (14, 15-16). The infection rate of Cryptosporidium among livestock in some rural parts of Korea was 94 % (17).

Our data indicated a potential risk of transmission of Cryptosporidium from animal to humans. Mojarad suggested that zoonotic transmission is the main mode of transmission of Cryptosporidium infection in Iran (18). One of the most important ways of contamination with Cryptosporidium spp. is contact with animals such as cattle, calves and sheep which are important reservoirs of this parasite (19). The prevalence of Cryptosporidium among the villagers in several area of Korea was 3.3% (17). Our research indicated that frequency of Cryptosporidium spp. among human in these areas was higher than the other rural region of Iran. The prevalence of the parasite in various parts of Iran was 4.1% in west, 7% in southeastern, 2.2% in south, 7.7% in north west, and 2.5% in central parts of Iran (20). We detected that Cryptosporidium spp. in children under 10 years was higher than others who were in contact with animals. This finding was confirmed by Joachim et al. (2). Cryptosporidium sp. infection has usually been found in children lived close to animals.

The present study indicates no significant difference between the infection rate of *Cryptosporidium* spp. and sex. Males have higher risk than females because they may expose to infections more than females. Our findings also showed that there was no significant difference between the infection rate of *Cryptosporidium* sp. Oocyst and clinical signs.

In winter we had the highest frequency of Cryptosporidium spp. in southwest Iran. There was significant association (P < .05)between seasons and infection rate of Cryptosporidium in animals. Several factors particularly rain may play an important role in formative disseminated of animal to Cryptosporidium. The most important factors in Cryptosporidium dispreading is rain and the food contamination. Environmental contamination with feces can increase diarrhea in calves and cattle by microorganisms and gastrointestinal parasites (21). In rainy seasons occurrence of Cryptosporidium was 6.3% and in dry seasons was 2.7% (22). We detected the highest *Cryptosporidium* spp. in turkeys as 62.5% in spring and the lowest 0% in summer. In some studies, C. meleagridis was found in turkey which signifies the great risk of infection due to closer contact of livestock with people who live enclose proximity to them. Evoy et al. described C. meleagridis an 'avian species' as a significant human pathogen suggested that turkey may play an important role in zoonotic Cryptosporidium transmission (23). The first infection of C.meleagridis in turkeys in Iran was reported by Meamar (24). In our studies we also found presence of Cryptosporidium oocyst in buffaloes. Cryptosporidium spp. have been detected in the feces of buffalos in Italy, Egypt, Cuba, India and Brazil (25).

Important factors in dissemination of the parasite are weather, location of sampling, infection dose and diversity of animal in area. Our study indicated that frequency of *Cryptosporidium* spp. infection in these areas was higher than the other rural regions of Iran and also animals could be an important source of infection in human.

### Acknowledgements

This research was supported by Vice Chancellor for Education of Tehran University of Medical Science. The authors wish to thanks Mr. M Pirestani and F.Tarighi for laboratory assistance. The authors declare that there is no conflict of interests.

## Reference

- Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int J Parasitol. 2000; 30(12-13):1305-22.
- Caccio SM, Cordoba MA . Molecular epidemiology of human cryptosporidiosis. Parassitologia. 2005; 47(2):185-92.
- Xiao L, Ryan UM. Cryptosporidiosis: an update in molecular epidemiology. Curr Opin Infect Dis. 2004; 17(5):483-90.
- Hazrati Tappeh 1 KH, Gharavi 2 MJ, Makhdoumi 3 K, Rahbar 1 M, Taghizadeh A. Prevalence of *Cryptosporidium* spp. Infection in Renal Transplant and Hemodialysis Patients. Iranian J Publ Health, 2006; 35(3):54-57.
- Meinhardt PL, Casemore DP, Miller KB. Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. Epid Rev. 1996; 18(2): 118-36.
- Mirzaei M. Prevalence of *Cryptosporidium* sp. infection in diarrheic and non-diarrheic humans in Iran. Korean J Parasitol. 2007; 45(2):133-7.
- 7. Markell E, John D, Krotoski W. Markell and Voge's Medical Parasitology (8th Edi-

tion).Philadelphia: W.B. Saunders Company, 1999.

- 8. Ridley DS, Hawgood BC. The value of formol-ether concentration of faecal cysts and ova. J Clin Pathol. 1956; 9(1):74-6.
- Henriksen SA, phhlenz JF. staining of *Cryptosporidia* by a modified Ziehl-Neelsens technique. Acta Vet Scand. 1981; 22: 594-6.
- 10. Azami M. Prevalence of *Cryptosporidium* infection in cattle in Isfahan, Iran. J Eukaryot Microbiol. 2007; 54(1):100-2.
- Gharagozlou1 MJ, Dezfoulian O, Rahbari S, Bokaie S, Jahanzad I, Razavi ANE. Intestinal Cryptosporidiosis in Turkeys in Iran. J Vet Med A. 2006; 53: 282 - 5.
- Fotouhi Ardakani R, Fasihi Harandi M, Solayman Banaei S, Kamyabi H, Atapour M, Sharifi I. Epidemiology of *Cryptosporidium* Infection of Cattle in Kerman/Iran and Molecular Genotyping of some Isolates. J of Kerman Univ Med Sci. 2008; 15(4):313-20.
- Keshavarz A, Haghighi A, Athari A, Kazemi B, Abadi A, Mojarad EN. Prevalence and molecular characterization of bovine *Cryptosporidium* in Qazvin province, Iran. Vet Parasitol. 2009; 160(3-4):316-8.
- Kumar D, Sreekrishnan R, DAS S.S. Cryprosporidiosis: an emerging disease of zoonotic importance. Proc Nat Acad Sci India. 2005; 75: p. 160-72.
- 15. O'Donoghue PJ. *Cryptosporidium* and cryptosporidiosis in man and animals. Int J Parasitol. 1995; 25: 139-95.
- Santín M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet Parasitol. 2004; 122: 103-17.
- 17. Yu JR, Lee JK, Min Seo M, Kim SI, Sohn WM, Huh S, Choi HY, Kim TS. Prevalence of cryptosporidiosis among the villagers and domestic animals in several rural areas of Korea. Korean J Parasitol. 2004; 42(1):1-6.
- Nazemalhosseini Mojarad E, Keshavarz A, Taghipour N, Haghighi A, Kazemi B, Athari A. Genotyping of *Cryptosporidium* spp. in clinical samples: PCR-RFLP analysis of the TRAP-C2 gene. Gastroenterology

and Hepatology from Bed to Bench. 2011; 4(1):29-33.

- Goh S, Reacher M, Casemore DP, Verlander NQ, Chalmers R, Knowles M, Williams J, Osborn K, Richards S. Sporadic cryptosporidiosis, North Cumbria, England, 1996-2000. Emerg Infect Dis. 2004; 10(6):1007-15.
- Keshavarz A, Athari A, Haghighi A, Kazami B, Abadi A, Nazemalhoseini Mojarad E, L Kashi . Genetic Characterization of *Cryptosporidium* spp. among Children with Diarrhea in Tehran and Qazvin Provinces, Iran. Iranian J Parasitol. 2008; 3(3): 30-6.
- Ayinmode AB, Fagbemi BO. Prevalence of *Cryptosporidium* infection in cattle from South Western Nigeria. Vet Archiv. 2010; 80(6):723-31.
- 22. Das P, Roy SS, Mitradhar K, Dutta P, Bhattacharya MK, Sen A, Gandipan S, Bhattacharya SK, Lal AA, Xiao L. Mole-

cular Characterization of *Cryptosporidium* spp. 1 in 2 children in Kolkata, India. J Clin Microbiol. 2006; 44(11):4246-9.

- McEvoy JM, Giddings CW. *Cryptosporidium* in commercially produced turkeys onfarm and postslaughter. Lett Appl Microbiol. 2009; 48:302-6.
- Meamar AR, Guyot K, Certad G, Dei-Cas E, Mohraz M, Mohebali M, et al. Molecular characterization of *Cryptosporidium* isolates from humans and animals in Iran. Appl Environ Microbiol. 2007; 73(3):1033-5.
- 25. Gomez-Couso H, Amar CF, McLauchlin J, Ares-Mazás E. Characterization of a Cryptosporidium isolate from water buffalo (Bubalus bubalis) by sequencing of a fragment of the *Cryptosporidium* oocyst wall protein gene (COWP). Vet Parasitol. 2005; 131(1-2):139-44.