Prevalence of *Cryptosporidium* Infection in Sheep in Iran

İran'da Koyunlarda *Cryptosporidium* enfeksiyonu prevalansı

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ABSTRACT

Objective: *Cryptosporidium* is an important zoonotic parasite in humans and animals worldwide. This study was conducted to investigate the prevalence of *Cryptosporidium* infection in Iran.
Methods: Fecal samples (n=1.749) were collected randomly in asymptomatic sheep from different rural regions of Iran in 2011 to 2012. All samples were examined by using the cold modified Ziehl-Neelsen staining technique.
Results: Oocysts of *Cryptosporidium* was found in 11.3% (198/1749) of samples (9.8<CI 95%<12.8). There was a statistical differences among *Cryptosporidium* infection, age groups (p<0.0001), and gender (p=0.02).
Conclusion: This study is the first report of *Cryptosporidium* infection in sheep in different regions of Iran. Therefore, further comprehensive molecular studies in sheep to identify the source of contaminations (animals or humans) and designing control strategies is highly recommended. *(Turkiye Parazitol Derg 2014; 38: 22-5)*

Key Words: *Cryptosporidium*, prevalence, sheep, Iran

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ÖZET

Amaç: *Cryptosporidium*, dünya çapında, insanlarda ve hayvanlarda önemli bir zoonotik parazittir. Bu çalışma İran'da *Cryptosporidium* enfeksiyonu prevalansını araştırmak amacıyla yapıldı.
Yöntemler: 2011-2012'de İran'ın farklı kırsal bölgelerinden asemptomatik koyunlardan rastgele dışkı örnekleri (n=1749) toplandı. Tüm örnekler soğuk modifiye Ziehl-Neelsen boyama tekniği kullanılarak incelendi.
Bulgular: *Cryptosporidium* oocyst'leri örneklerin %11,3'ünde (198/1749) bulundu (9,8<CI 95%<12,8). Cinsiyet (p=0,02) ve yaş grupları arasında (p<0,0001) *Cryptosporidium* enfeksiyonu açısından istatistiksel farklılık mevcuttu.
Sonuç: Bu çalışma, İran'ın farklı bölgelerinde koyunlarda *Cryptosporidium* enfeksiyonu hakkında ilk rapordur. Bu nedenle, kontaminasyonların (hayvanlar veya insanlar) kaynağını belirlemek ve kontrol stratejilerini tasarlamak için koyunlarda daha kapsamlı moleküler çalışmalar şiddetle tavsiye edilir. *(Turkiye Parazitol Derg 2014; 38: 22-5)*

Anahtar Sözcükler: *Cryptosporidium*, prevalans, koyun, İran

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INTRODUCTION

Cryptosporidium is an important zoonotic parasite in humans, domestic and wild animals such as mammals, birds, reptiles and fish (1). The distribution of this parasite is worldwide, and commonly is found in warm and wet seasons (2, 3).

Cryptosporidium was first described affecting in mice by Tyzzer in 1907 (4). In sheep, cryptosporidiosis was first described in lambs with diarrhea in Australia in 1974 (5). Also, cryptosporidiosis was first found in a native rooster in Iran in 1985 (6).

A single oocyst is sufficient to produce infection in susceptible hosts. Oocysts are most commonly transmitted by the fecal-oral route through direct host-to-host contact, and indirect contamination of food or water; although aerosol transmission of oocysts has also been reported (7). Zoonotic transmission has been confirmed by epidemiological investigations such as pets, farm animals and by accidental infection of veterinary workers (3, 7-9).

Cryptosporidiosis is commonly self-limiting in the immunocompetent hosts (7). Young animals appear to be more sensitive to disease, while infections in adult animals are often asymptomatic or do not occur (1, 7, 10). In sheep, cryptosporidiosis is presented as a mild to severe yellowish liquid diarrhea with a strong odor, weight loss, depression, abdominal pain, and death usually involving animals up to one month of age (7, 11, 12).

Cryptosporidiosis may have significant economic losses in animal husbandry. Additionally, infected animals pose a health risk to humans given the zoonotic potential of this parasite, which continues to cause a serious and life-threatening disease in immunodeficient patients such as AIDS (1, 7).

Different methods have been developed to diagnosis of *Cryptosporidium*. The most common method of them involves oocysts detection using fecal smears examination with Ziehl-Neelsen staining (7, 13). The molecular techniques have been also used to identify *Cryptosporidium* in feces of sheep in some countries (12, 14).

There is little information regarding the occurrence of *cryptosporidium* infection in sheep and other hosts in different regions of Iran (8, 9, 15, 16). Also, there is no published comprehensive information of *Cryptosporidium* prevalence in sheep in this area.

The principal aim of current study was to obtain the prevalence of *Cryptosporidium* infection in sheep in Iran.

METHODS

Study Area

Iran lies between latitudes 24° and 40° N, and longitudes 44° and 64° E is located in Middle-East, Asia. It covers an area of 1,648,195 km^2 by different climate ranges including cool mountainous areas in West, rainfall and temperate plains in North, arid and desert in Central, and tropical area in South of Iran. This country is economically important for crops and animal husbandry, such as sheep breeding. In Iran, the sheep population is estimated to be at 50 millions according to Iranian Veterinary Organization (IVO) report in 2010.

Sample Collection and Examination

1,749 stool samples were collected randomly in sheep without clinical signs in different rural regions of Iran from 2011 to 2012 in cross-sectional study; kept under the semi-intensive feeding system (Table 1). A sample was taken from the rectum by using a disposable plastic bag for each animal, which were fixed quickly in 10% formalin until the examination. All samples were concentrated by formalin-ether technique and examined for microscopy (100X) using cold modified Ziehl-Neelsen staining (3).The *Cryptosporidium* oocysts were observed as spherical red colored objects, around 4-5µm in diameter, with internal structures somehow crescent shape (3).

Statistical analysis

Chi-square test (X^2) was used to compare infection rates between different age groups and gender. Odds ratios (OR), confidence interval (CI), X^2 and *p*-value were calculated separately for each variable. *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Oocysts of *Cryptosporidium* was found in 11.3% (198/1749) of samples (9.8<CI 95% <12.8). There was a statistical differences among *Cryptosporidium* infection, age groups (x^2=23.929, p<0.0001, DF=1, OR=2.1), and gender (x^2=5.385, p=0.02, DF=1, OR=1.4). The detailed information of different regions is summarized in Table 1-3.

DISCUSSION

Cryptosporidiosis is a common cause of diarrhea in humans and animals worldwide (7). The economic losses were incurred due to *Cryptosporidium* infections in livestock and the threat to human health are major concerns. Therefore prevention and control measures need to be adopted and regulated in the animal environment. Veterinarians have an important role to recog-

Table 1. Prevalence of Cryptosporidium infection in different location

	Study area						
	A	B	C	D	E	F	Total
No. of sample	347	300	184	300	300	318	1749
Positive%	9.5	19	12.5	6.3	3.7	17.3	11.3
CI 95%	6.5-12.5	14.6-23.4	7.7-17.3	3.6-9	1.6-5.8	13.2-21.4	9.8-12.8

A: Hamedan province, West of Iran; B: Esfahan province, Central of Iran; C: Yazd province, Central of Iran; D: Fars province, South of Iran; E: Bushehr province, South of Iran; F: Mazandaran province, North of Iran

Table 2. Prevalence of *Cryptosporidium* infection in different location

Age groups (year)	Study area No. of sample (Positive %)						
	A	B	C	D	E	F	Total
≤1	132 (13.6)	138 (23.9)	19 (21.1)	114 (0.88)	52 (17.3)	100 (19)	555 (16.7)
>1	215 (6.98)	162 (14.8)	165 (11.5)	186 (4.8)	248 (0.8)	218 (16.5)	1194 (8.8)
p-value	0.04	0.04	0.233	0.174	<0.0001	0.586	<0.0001

A: Hamedan province, West of Iran; B: Esfahan province, Central of Iran; C: Yazd province, Central of Iran; D: Fars province, South of Iran; E: Bushehr province, South of Iran; F: Mazandaran province, North of Iran

Table 3. Prevalence of *Cryptosporidium* infection in different gender groups

Gender	Study area No. of sample (Positive %)						
	A	B	C	D	E	F	Total
Male	125 (8.8)	70 (24.3)	34 (17.6)	73 (9.6)	37 (5.4)	116 (18.9)	455 (14.3)
Female	222 (9.9)	230 (17.4)	150 (11.3)	227 (5.3)	263 (3.4)	202 (16.3)	1294 (10.3)
p-value	0.735	0.197	0.314	0.189	0.547	0.55	0.02

A: Hamedan province, West of Iran; B: Esfahan province, Central of Iran; C: Yazd province, Central of Iran; D: Fars province, South of Iran; E: Bushehr province, South of Iran; F: Mazandaran province, North of Iran

nize emerging disease and apply strategies to prevention and control of infections (17).

To increase our knowledge of the parasite's epidemiology, biology, taxonomy, and molecular diversity, extensive research is needed, as are improved detection protocols capable of differentiating species and genotypes. Understanding the biological behavior and correctly identifying the offending *Cryptosporidium* spp. will be critical if the intervention and control strategies are to be effective (7).

The prevalence of *Cryptosporidium* infection was reported 4% to 85% in sheep worldwide (18). Previous investigations conducted on the prevalence of *Cryptosporidium* in sheep based on microscopy have reported prevalences ranging from 0% in Ethiopia, 2.6% in Autralia, 3.7%-47% in Brazil, 13.6%-46.5% in Turkey, 25.7% in Mexico, 29% in Greece, and 42.1% in Serbia (1, 2, 10, 12, 13, 17, 19, 20-22). In Iran, this rate was reported separately 4% in North, 13.8% in Central, 2.5% and 8.6% in West, 9.2% and 28.6% in Capital regions (3, 8, 9, 18, 23).

In this study, the infection rate was partly similar to the study done in Poland (10.1%) and partly different to studies taken in the other countries (24). Also, infection rate in rainfall area (17.3% in Mazandaran province) was higher than the other regions (Table 1); it is agreement consistent with previous reports (2). Different of hygiene conditions and management in farms, study design, methods, climates and different geographical regions may be the main cause of varied results (1, 7).

In our study, the infection rate in ≤1 yr animals (16.7%, p<0.000l, OR=2.1) was found higher than >1 yr (8.8%); the results are consistent with those of other researchers reporting a strong correlation (p<0.05) between the age and infection (1, 8-10, 12, 17-19, 24). In contrast to finding of this study, there was a close association (p>0.05) between infection and age groups in asymptomatic adult sheep from Mexico (13).

Age is the major risk factor in spreading of cryptosporidiosis (19); and risk of infection and morbidity are greater in neonatal animals (21). Increasing prevalence rate in low age groups may be due to immature immune system and their sensitivity against infection (18).

In this study, infection rate was reported 14.3% in male (p=0.02, OR=1.4) and 10.3% in female animals; the finding is opposite to the other investigations (3, 8, 9, 23).

In current study, *Cryptosporidium* infection was asymptomatic in all animals. The prevalence of *Cryptosporidium* in healthy sheep implies that they can serve as reservoirs of the infection (21).

In Iran, the most sheep breeding farms are traditional and the sheep have direct contacts with other animals. It is also possible that the quality of zoohygienic conditions of animal husbandry and grazing practices influences the exposure of animals to cryptosporidial infection.

Due to the great morphological similarity among species of *Cryptosporidium*, utilizing of microscopic observation of oocysts alone is not sufficient for identification of species (12).

CONCLUSION

This study is the first report of *Cryptosporidium* infection in sheep in different regions of Iran. Therefore further comprehensive molecular studies in sheep cryptosporidiosis to identify the source of contaminations (animals or humans) and designing control strategies is highly recommended.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Bu-Alisina university, faculty of Paraveterinary (2010-207).

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