



Original Article

A survey on prevalence rate of *Cryptosporidium* infection in stockmen and sheep from Bukan, Iran

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ABSTRACT

Cryptosporidium is a protozoan that causes an important opportunistic infection in humans and ruminants. The purpose of this study is to investigate the prevalence of cryptosporidiosis infection based on some epidemiological factors in stockmen and sheep in Bukan county. In this study, conducted from July to December 2018, 218 fecal samples were collected from sheep and 200 fecal samples from stockmen in Bukan county, Iran. After preparation, the samples were stained using the Modified Ziehl-Neelsen stain method and examined microscopically at 100X and 40X magnification. In total, six stockmen (3%) and 25 sheep (11.46%) were diagnosed as infected with *Cryptosporidium*. In the present study, the highest prevalence was observed with a significant statistical difference in the age group of 1-7 years (20.83%), among people with clinical symptoms (26.32%), and among those who consumed home-grown vegetables cultivated with animal manure (18.52%) ($p < 0.05$). No significant statistical difference was observed in other studied parameters ($p > 0.05$). Additionally, there is a statistically significant relationship between the level of infection in sheep with *Cryptosporidium* and their age group ($p < 0.05$). Further investigations revealed a statistically significant relationship between the sex of sheep and the infection in Bukan county ($p < 0.05$).

بررسی میزان شیوع آلودگی به کریپتوسپوریديوم در دامداران و گوسفندان شهرستان بوکان، ایران

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چکیده

کریپتوسپوریديوم تک یاخته ای است که موجب عفونت فرصت طلب مهمی در انسان و نشخوارکنندگان می شود. هدف از این مطالعه بررسی میزان شیوع عفونت کریپتوسپوریديوزیس بر اساس برخی فاکتورهای اپیدمیولوژیک در دامداران و گوسفندان شهرستان بوکان است. در این مطالعه که از تیرماه تا آذر ماه ۱۳۹۸ انجام شد، ۲۱۸ نمونه مدفوعی از گوسفندان و ۲۰۰ نمونه مدفوعی از دامداران شهرستان بوکان، ایران جمع آوری شد. پس از تهیه گسترش، گسترش های حاصل با روش ذیل نلسون تغییر یافته رنگ آمیزی شدند و مورد بررسی میکروسکوپی با بزرگنمایی ۱۰۰× و ۴۰× قرار گرفتند. در مجموع تعداد ۶ نفر (۳٪) از دامداران و تعداد ۲۵ مورد مثبت (۱۱/۴۶ درصد) از گوسفندان مورد مطالعه آلوده به کریپتوسپوریديوم تشخیص داده شدند. در مطالعه حاضر، بیشترین میزان شیوع با تفاوت آماری معنی دار در گروه سنی ۱-۷ سال (۲۰/۸۳٪)، افراد دارای علائم بالینی (۲۶/۳۲٪) و افرادی که مصرف کننده سبزیجات خانگی پرورش یافته با کود دامی بودند (۱۸/۵۲٪) مشاهده شد ($p < 0.05$) و در مورد سایر پارامترهای مورد مطالعه، تفاوت آماری معنی داری مشاهده نگردید ($p > 0.05$). همچنین، بین میزان آلودگی گوسفندان به کریپتوسپوریديوم و گروه سنی رابطه آماری معنی داری وجود داشت ($p < 0.05$). در ادامه بررسی ها مشخص شد که بین جنسیت گوسفندان و عفونت شهرستان بوکان رابطه آماری معنی داری وجود دارد ($p < 0.05$).

واژه های کلیدی: کریپتوسپوریديوم، گوسفند، دامدار، بوکان، ایران.

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INTRODUCTION

Cryptosporidium protozoa are members of the *Apicomplexa* phylum, and 12 species of *Cryptosporidium* are known to parasitize animals and humans. This protozoan was first reported by Tyzzer in 1907 from the gastric glands of a laboratory mouse [1]. *Cryptosporidium* is one of the most important common intestinal pathogens reported in many organisms and in most parts of the world [2]. Because this protozoan is a common parasite between humans and animals, many reports of transmission between humans and animals have been documented worldwide [1][3-5][6-10]. Epidemiological studies have shown that ruminants and dogs are sources of protozoan infections transmitted to humans [4]. This parasite is of special importance in terms of health in humans and economically in animals and breeders, as it can cause severe and even fatal gastroenteritis. It can also cause respiratory diseases in children, adults, AIDS patients, and animals with compromised immune systems [11]. The infection in humans was first reported in 1976. Since then, this parasite has been reported in one to four percent of patients with diarrhea in developing countries and up to 16 percent in less developed countries, especially those with less sanitary drinking water. This infection is common in children, and it seems that most adults are immune to it. However, it is of strategic importance in people with immune system defects, especially those with diseases such as diabetes, AIDS, various neoplasia, and those who need organ transplants. As the disease appears more frequently, it seems that the prevalence of this parasite will be a concern in the future due to advancements in diagnostic methods and population growth. Based on studies, the infection rate of sheep with *Cryptosporidium* in Iran and other countries ranges from 4-85%. In a study conducted by

Heydari and Qarakhani in 2012, the rate of *Cryptosporidium* infection in asymptomatic sheep in Iran was 11.3%, with the highest rate of contamination in Mazandaran at 17.3% and the lowest rate in Bushehr at 3.7% [12, 13]. Constant monitoring of this protozoan in human societies, especially those at double risk due to occupational reasons, and in animals as an opportunistic zoonosis agent, in the water and food sources of humans and animals, is one of the most important strategies defined in management. The aim of the present study is to investigate the prevalence of *Cryptosporidium* infection in cattle breeders and sheep in Bukan county.

MATERIALS AND METHODS

Study area

The present study was conducted in different rural areas around Bukan county, West Azerbaijan province. From July to December 2018, 218 stool samples from sheep were collected in five stages, as well as 200 stool samples from stockmen. Special containers were provided for stool samples, and personal consent was obtained due to the fact that the test was free. Samples were collected by mentioning individual characteristics.

Sample size

In this study, 218 sheep in different age groups (less than one year, between one to two years, two to three years, three to four years, and over four years) and 200 stockmen aged 1 to 77 years were randomly selected. During this study, the fecal samples of stockmen and sheep were prepared.

Parasitological procedures

After preparation, the stool samples were placed in sterile urine containers. After filling in the characteristics, sampling date, and age and genus of the stockmen, they were transferred to the laboratory for preparation. A direct smear was prepared by placing a drop of physiological serum on the slide and dissolving some of the fresh feces with a plastic applicator. Then, 1-2 grams of feces were transferred to a test tube containing 12 ml of distilled water and centrifuged at 1500 RPM for 5 minutes. After centrifugation, the top solution was discarded, and only the residual sediment remained. The tubes were then filled with Scheathers sugar solution (500 grams of sugar dissolved in 320 milliliters of water, with 6.5 grams of phenol added). The test tube was centrifuged at 500 RPM for 15 minutes, and the upper part of the tube was gently removed with a metal loop and transferred to the slide. After drying the smear

second stage of sampling, 43 slide samples were again prepared with a swab from the membrane of the inner rectal wall of diarrheal cases, and stool samples were stained with the same method. In the third stage, 46 samples were collected, and in the next stages, 43 samples were collected, and the expansion was prepared. After drying, the slides were examined for false particles with a 100X oil lens. In this staining method, *Cryptosporidium* oocytes were observed with 100X and 40X objective lenses, which are round or oval bodies with red color on a green background, measuring 4-6 microns. The basis for diagnosing *Cryptosporidium* oocysts in this method is to see sporozoites inside the oocysts (Figure 1). To check the slides, the whole slide was first checked, and then the degree of contamination was shown as (+), (++), and (+++) in five fields.

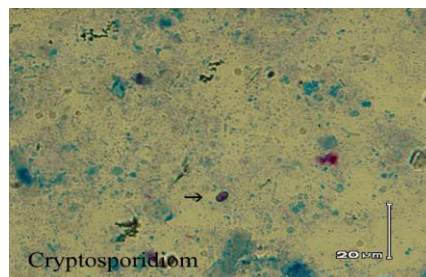


Figure 1: Image of *cryptosporidium* oocyst with the modified Ziehl Neelsen stain.

in the open air, it was fixed by passing the slide over a flame three times. The prepared samples were then stained using the Modified Ziehl-Neelsen stain method. Regarding the fecal samples of sheep, in the first stage, 43 diarrheal samples were prepared and passed through two layers of sterile gauze in the laboratory. They were then centrifuged at 2500 RPM for 5-10 minutes. After discarding the supernatant, the obtained uniform sediment was placed on a glass slide with the help of another glass slide and dried at laboratory temperature. After drying, the slides were stained with the Modified Ziehl-Neelsen stain method. In the

Statistical analysis

For data analysis, descriptive statistics with 95% confidence intervals (95% CI) were used, and logistic regression was used to determine the effect of the mentioned risk indicators (age, sex, etc.) on the prevalence of infection. Data were analyzed using Stata, version 11.2.

RESULTS

In this study, 218 sheep and 200 stockmen were sampled in Bukan county, Iran. In total, six

stockmen (3%) were diagnosed as infected with *Cryptosporidium*. The samples were evaluated based on age, sex, place of residence, appearance of clinical symptoms, direct contact with animal feces, frequent hand washing, consumption of domestic vegetables grown with animal manure, how vegetables were washed, and the type of water consumed. The studied samples were placed in 11 age groups with a 7-year class interval. The results of Table 1 show the highest level of infection in the age group of 1-7 years, with five positive cases and 20.83% infection in the population of stockmen. The highest level of contamination based on the appearance of clinical symptoms is 26.32% (Figure 2), and based on the consumption of homegrown vegetables with manure, it is 18.52% (Figure 3). Data analysis shows a statistically significant relationship in relation to age, presence of clinical symptoms, and consumption of domestically grown vegetables with animal feces ($p < 0.05$). However, it does not show a statistically significant relationship in relation to sex, place of residence, direct contact with animal feces, frequent hand washing, how consumed vegetables were washed, and type of water consumed ($p > 0.05$).

Among the examined sheep, 25 positive cases (9.16%) were identified. According to Table 2, the frequency of sheep samples in Bukan county was examined in five age groups. There were 43 samples and nine positive cases in the population under 1 year of age, with 15.64% infection in the age group population and 3.30% compared to the total population of sheep (218 heads). In the age group between 1-2 years, 43 samples were taken, of which six were positive. The percentage of infection in the age group population is 10.66% and 2.2% of the total number of sheep. In the age group of 2-3 years, among the population of 46 sheep studied, four positive cases were recorded with 6.4% compared to the age group population and 1.46% compared to the total population of sheep. In the age group of 3-4 years, four positive cases were observed among the 43 samples; the infection percentage was 8% among the age group population, and the infection was 1.46% among the total population of sheep. In the age group above 4 years, two positive cases were recorded with 4.32% infection and 0.73% of the total infected sheep population. Data analysis shows a statistically significant relationship with the age of sheep

Table 1. Frequency and prevalence of *Cryptosporidium* by age groups in stockmen of Bukan county, Iran

Age group	Positive number	Percentage (%)	Negative number	Percentage (%)	Total	Percentage (%)
1-7	5	20.83	19	17.79	24	12.31
8-14	0	0.00	22	100.00	22	11.28
15-21	1	3.13	31	96.88	32	16.41
22-28	0	0.00	30	100.00	30	15.38
29-35	0	0.00	29	100.00	29	14.87
36-42	0	0.00	19	100.00	19	9.74
43-49	0	0.00	9	100.00	9	4.62
50-56	0	0.00	11	100.00	11	5.64
57-63	0	0.00	13	100.00	13	6.67
64-70	0	0.00	10	100.00	10	5.13
70-77	0	0.00	1	100.00	1	0.51
Total	6	3.00	194	97.00	200	100.00

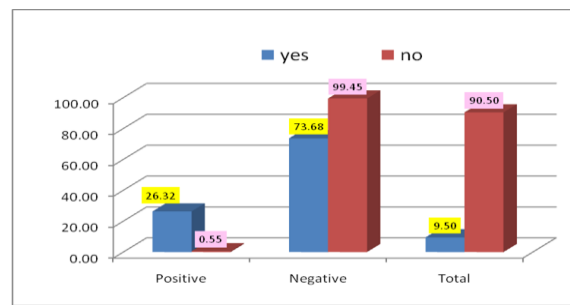


Figure 2: Frequency and prevalence of *Cryptosporidium* by clinical symptoms in stockmen of Bukan county, Iran

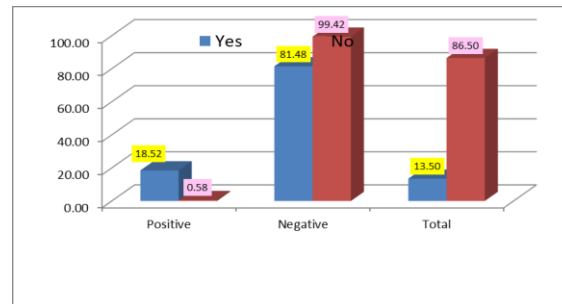


Figure 3: Frequency and prevalence of *Cryptosporidium* by fecal consumption of home-grown vegetables in stockmen of Bukan county, Iran

Table 2. Frequency and prevalence of *Cryptosporidium* by age group in the sheep population of Bukan county, Iran

Age group	Number	Positive number	Total percentage (%)	Total percentage by species (%)
X<1	40	9	15.64	3.30
1-2	45	6	10.66	2.2
2-3	49	4	6.4	1.46
3-4	41	4	8	1.46
X>4	43	2	4.32	0.73
Total	218	25	-	9.16

Table 3. Frequency and prevalence of *Cryptosporidium* by sex in the sheep population of Bukan county, Iran

Sex	Number	Positive number	Total percentage (%)	Total percentage by species (%)
Male	34	4	1.46	9.40
Female	184	21	7.70	9.12
Total	218	25	9.16	

($p < 0.05$). According to Table 3, out of 218 sheep studied, 34 were male and 184 were female. Four positive cases were observed in the male sheep population, and 21 positive cases were observed in the female sheep population. The percentage of infection rate according to the sex of sheep is 9.40% for male sheep and 9.12% for female sheep. The percentage of contamination in relation to the total population is 1.46% for males and 7.70% for females. Data

analysis shows a statistically significant relationship between the sex of sheep and the percentage of contamination in the total population of sheep ($p < 0.05$).

DISCUSSION

The disease caused by *Cryptosporidium* is considered a zoonosis [14] and is an intracellular, extracytoplasmic protozoan [15].

Nineteen species of this protozoan have been identified from different hosts, including reptiles, birds, and mammals [1]. This parasite is the cause of endemic infections and travelers' diarrhea. The frequency pattern of the disease has been explained based on age, season, geographical location, and transmission routes. This infection sometimes causes death in stockmen and is therefore clinically and economically important [16]. In humans, the severity and duration of the disease depend on the host's immune system. The groups at risk are children, kindergarten personnel, farmers, stockmen, and those who work in care centers and hospitals. Traveling from developing countries to developed countries can be considered a risk factor in the spread of *Cryptosporidium* [16]. Human infection with *Cryptosporidium* protozoan in different reports from different countries of the world, depending on climatic conditions, geography, and demographic factors, includes different figures. The same principle also exists in the cities of Iran, with varying prevalence rates reported for different geographical regions of the country, such as Shiraz (3.5%), Ahvaz (2.23%), Rasht (1.57%), Qazvin (4.5%), Hamadan (5.3%), Isfahan (16.9%), Mashhad (14.6%), Urmia (10%), and Tabriz (6.15%) [5]. In general, *Cryptosporidium* infection was diagnosed in six people (3%) of the studied population, which seems to be lower compared to the general figures reported for normal people. This finding is similar to the results reported by Jafari (2012), where infection was observed in only 0.87% of people in rural areas around Hamedan in connection with stockmen [13]. It seems that obtaining such a result indicates the small role of this occupation in acquiring infection in the studied area, which is probably due to the role of the immune system in the establishment of infection in those frequently in contact with protozoa, or due to high awareness and appropriate health conditions of the studied

people. The prevalence of infection in people who lived in urban or rural areas did not have a statistically significant difference, which was also reported in the research of Ranjbar et al. (2011) ($p < 0.05$) [9]. The prevalence of infection in people who were in direct contact with animal feces compared to those who did not have direct contact showed no statistically significant difference, which was also reported in Jafari's study (2012) ($p < 0.05$) [13]. This issue highlights that contact with feces in this group, regardless of the duration of contact, is not at zero level like normal people, and this problem causes different results from those reported by Naserifar et al. (2008). Reaching a threshold of contact with animal feces may be decisive for protozoan infection [22]. Likewise, the role of the immune system, which can create better responses by repeated exposure to protozoa, should not be overlooked. Regarding the parameter of frequent hand washing, the observed statistical difference was not significant ($p < 0.05$), which contradicts the report of Nouri et al. (1991), which showed a higher prevalence in shepherds compared to villagers who have more access to soap and water for hand washing [8]. The possible reason for such a case may be due to the lack of significant difference in hand washing among the stockmen studied compared to the difference between shepherds and villagers in the aforementioned study. The age group of 1 to 7 years has the highest prevalence rate (20.83%) among the age groups, which is consistent with the results of many researchers ($p < 0.05$) [17]. Regarding the sex parameter, no significant difference was observed between the two sexes in this research ($p < 0.05$), which contradicts most of the research conducted on ordinary people [17, 18]. The reason for this is probably related to the fact that in normal people, contact with animals or soil or any source of oocysts due to work or lower health behaviors is more common in males than in females, but as

mentioned earlier, in the herdsmen, they probably reach the threshold of infection, and the role of the immune system is the same for both sexes. The significant difference in the presence of protozoa in people with clinical symptoms attributed to cryptosporidiosis (26.32%) and people without symptoms (0.55%) is a relatively common issue and is also observed in the reports of other researchers ($p < 0.05$) [6, 8, 19]. One of the desired parameters of this study was the use of vegetables grown at home using animal manure, which showed a significant difference in the prevalence of those who consume these vegetables (18.52%) compared to those who do not consume such vegetables (0.58%) ($P < 0.05$). Cultivation of vegetables using the manure of animals grown in the same house or area is considered a potentially dangerous source for the spread of infection [7]. However, since proper washing of vegetables for raw consumption can decisively reduce the contamination potential of these vegetables, and all the contaminated individuals studied in this research only used water to wash vegetables, obtaining such a result is completely expected ($p < 0.05$). Based on studies, the infection rate of sheep with *Cryptosporidium* in Iran and other countries is 4-85%. In a study conducted by Heydari and Qarakhani in 2012, the rate of *Cryptosporidium* infection in asymptomatic sheep in Iran was 11.3%, with the highest rate of contamination in Mazandaran at 17.3% and the lowest rate in Bushehr at 3.7% [12, 13]. Also, in a study conducted by Fasihi Harandi and Fatuhi Ardakani in 2017 on the sheep of Kerman, they reported 13.8% *Cryptosporidium* infection in the sheep of this county [2]. The present study was conducted with the aim of investigating the prevalence of *Cryptosporidium* infection in sheep in Bukan County, Iran. The infection rate of sheep is 9.16%. The results of this study are in line with other research [2, 11, 20, 21]. In relation to the age of the studied

sheep, the highest level of infection was observed in the age group under 1 year, with 15.64% compared to the age group population and 3.30% compared to the total statistical population. As a result, there is a statistically significant relationship between the level of infection of sheep with *Cryptosporidium* and the age group ($p < 0.05$). In a study conducted by Khodri et al. in Kurdistan in 2012, they reported the highest level of contamination in lambs less than 1 month old [22], which could be due to the lack of development of the stockmen's immune system and the sensitivity of young stockmen against infections. In the following investigations, it was found that there is a statistically significant relationship between the sex of sheep in Bukan county ($p < 0.05$). The level of contamination in the population of females was more than that of males. Fashihi and Ardakani stated that the probability of infection in females is 62% higher than that of males [2]. However, in the study by Kasmore et al. in 1997, there is no significant relationship between the level of pollution and sex in humans and animals ($p < 0.05$) [23].

CONCLUSION

Regarding the level of contamination to cryptosporidiosis of stockmen in Bukan county, it seems that this segment of society has a lot of contact with animals such as cows and sheep, especially young animals. As required, performing activities such as the permanent collection of animal excrement and using it as agricultural fertilizer and house fuel increases the probability of getting involved with this protozoan. Also, the statistical analysis in this research regarding the level of contamination in the age groups of sheep is in line with the studies of other researchers, but in terms of the level of contamination based on sex, it is not consistent with other studies, which is the

reason for taking more samples in the population of female sheep.

ETHICS

Approved.

CONFLICT OF INTEREST

None.

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