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Prevalence of *Cryptosporidium spp.* Infection among Domestic Animals in Shiraz, Fars Province, Iran (2023)

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ABSTRACT

Background and Aim: *Cryptosporidium spp.* are parasitic protozoa that can cause cryptosporidiosis, leading to diarrhea, particularly in developing countries. While healthy individuals may have mild or asymptomatic infections, those with weakened immune systems can experience severe illness. This study aimed to determine the prevalence of *Cryptosporidium spp*. in domestic animals, including cattle, sheep, and horses, in Shiraz County, Fars province, Iran.

Materials and Methods: The fecal samples (n=189) were collected from cattle, sheep, and horses in Shiraz County focusing on both diarrheal and non-diarrheal animals during 2023. Microscopic methods were employed to assess the prevalence of *Cryptosporidium spp*. in samples. SPSS version 21 was used for the statistical analysis, applying Fisher's exact test to evaluate the association between diarrhea and *Cryptosporidium* infection (P<0.05).

Results and onclusion: The prevalence of *Cryptosporidium* in 189 fecal samples was found to be 22.7%. Cattle had the highest rate at 30.16%, while sheep and horses had lower rates of 20.63% and 17.46%, respectively. This study highlights the significant prevalence of *Cryptosporidium* in domestic animals in Shiraz County, emphasizing the need for ongoing surveillance and management to mitigate zoonotic transmission risks in Animal Husbandry settings.

Keywords: *Cryptosporidium spp.*, Domestic Animals, Microscopic Methods, Shiraz

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1. Introduction

Cryptosporidium species are single-celled parasitic protozoa capable of infecting various vertebrate hosts, including humans (1). *Cryptosporidiosis*, caused by *Cryptosporidium spp.*, often leads to diarrhea, especially in developing countries. In healthy individuals, it may be asymptomatic or cause self-limiting diarrhea with nausea, vomiting, headache, mild fever, and dehydration (2). In individuals with compromised immune systems, particularly those with acquired immunodeficiency syndrome (AIDS), the symptoms of infection can be severe, leading to profuse diarrhea and ultimately resulting in patient mortality (3). *Cryptosporidium spp.* is a major cause of diarrhea in ruminants (e.g., cattle, sheep). Humans can contract it through direct contact with infected people or animals, contaminated water or food, or even airborne transmission (4). *Cryptosporidiosis* has

been reported on all six continents, with the majority of cases occurring in developed countries among adults and children who sought laboratory testing due to gastrointestinal issues **(5)**. The most common species infecting humans are *Cryptosporidium parvum* and *Cryptosporidium hominis*; however, species such as *Cryptosporidium meleagridis*, *Cryptosporidium canis*, and *Cryptosporidium felis* have also been reported from various animal hosts **(6)**. Species like *Cryptosporidium ryanae*, *bovis*, and *andersoni* are regarded as non-zoonotic and exclusive to cattle. Sheep are also primarily infected with *Cryptosporidium xiaoi* and *Cryptosporidium ubiquitum* **(7)**. There is limited information regarding the prevalence of *Cryptosporidium* in horses; however, several molecular studies conducted in the United States, Italy, New Zealand, and the United Kingdom indicate that horses may serve as potential sources of human cryptosporidiosis infections, either directly or through watershed areas **(8)**. A systematic review and meta-analysis by Haghi et al **(9)** encompassing 100 studies from Iran, revealed varying prevalence rates of *Cryptosporidium* among different animal species, with rodents exhibiting the highest (20.8%) and dogs the lowest (4.9%) infection rates **(9)**. Therefore, cattle, sheep, and horses should be considered as sources of cryptosporidiosis infections for humans, both through direct contact and environmental contamination **(8)**. This study aimed to determine the prevalence of *Cryptosporidium* in domestic animals, including cattle, sheep, and horses, in Shiraz County, located in Fars Province, utilizing microscopic methods.

2. Materials and Methods

2.1 Population under Investigation

Sampling was done by visiting dairy farms, livestock establishments, and horse breeding facilities in various regions of Shiraz County from March 2023 and continued until May 2023. Cattle, sheep, and horses, both with and without diarrhea, were examined. Random sampling was performed by monitoring animals while they were defecated and 15 to 20 gr of fresh feces were collected. All samples were then placed in specialized fecal sample containers and transferred to laboratory. A total of 189 fecal samples were collected (63 samples from each species). Among these, 81 animals were male and 108 were female.

2.2 Concentration of Samples and Microscopic Evaluation

All collected fecal samples were placed in 50 mL falcon tubes and transported to the Intestinal Protozoa Laboratory at Shiraz University of Medical Sciences, Shiraz, Iran. They were promptly stored in an equal volume of physiological normal saline after collection and maintained at 4°C in a refrigerator until analysis.

Cryptosporidium spp. oocysts were purified using the saturated sugar floatation technique. Approximately, 3 gr of each sample was diluted with physiological saline, and after passing through a four-layer gas-tight filter, the coarse materials were separated, resulting in a homogeneous solution. The solution was centrifuged at 3000 g for 10 min. After discarding the supernatant, a sucrose solution with a specific gravity of 1.27g/cm^3 was added to the sediment at the bottom of the tube, and the resulting mixture was centrifuged at 2000 g for an additional 10 min. Finally, the oocysts floated to the surface of the solution were collected using a pipette and placed on a microscope slide to prepare a smear.

For staining, the Ziehl-Neelsen method (acid-fast staining) was employed. The samples prepared with methanol were initially fixed and subsequently stained using carbol fuchsin. Subsequently, the samples were washed with distilled water and decolorized using 3% acid-alcohol. Finally, the samples were stained with methylene blue for 1 min and examined under a 100x oil immersion lens after drying **(10)**.

2.3 Statistical Analysis

Statistical analysis was conducted using SPSS version 21. The Fisher's exact test was employed to assess the relationship between the presence or absence of diarrhea in the samples and the level of *Cryptosporidium spp.* infection. The P value less than 0.05 was considered significant.

3. Results and Discussion

3.1 Microscopic Evaluation Results

A comprehensive study was conducted involving the collection of 189 fecal samples from cattle, sheep, and horses in Shiraz County area. The primary objective was to assess the prevalence of *Cryptosporidium* among these animal populations. The overall prevalence of *Cryptosporidium spp.* across the collected samples was found to be 22.7%, indicating a significant level of infection within these livestock. Figure 1 shows representative *Cryptosporidium spp.* oocysts in fecal smears stained by Ziehl-Neelsen. When examining the data in more detail, the highest level of *Cryptosporidium* contamination was identified in cattle, with a prevalence rate of 30.16%. This suggests that cattle may serve as a critical reservoir for this pathogen, potentially influencing its transmission dynamics within the agricultural ecosystem. In comparison, sheep exhibited a lower prevalence rate of 20.63%, while horses had the lowest

contamination level at 17.46%. These findings highlight varying susceptibility and potential role of different livestock species in epidemiology of *Cryptosporidium* infections.

Additionally, the analysis revealed a notable correlation between the presence of diarrhea and the prevalence of *Cryptosporidium*. Specifically, the highest contamination rate of 32.47% was observed in fecal samples from animals exhibiting diarrheal symptoms (Table 1). The findings demonstrated that prevalence of *Cryptosporidium* infection was significantly higher in animals exhibiting symptoms of diarrhea. Additionally, the prevalence of infection was significantly higher in male animals compared to females. This significant association emphasizes the importance of monitoring and addressing *Cryptosporidium* infections, particularly in animals displaying clinical signs of gastrointestinal distress.

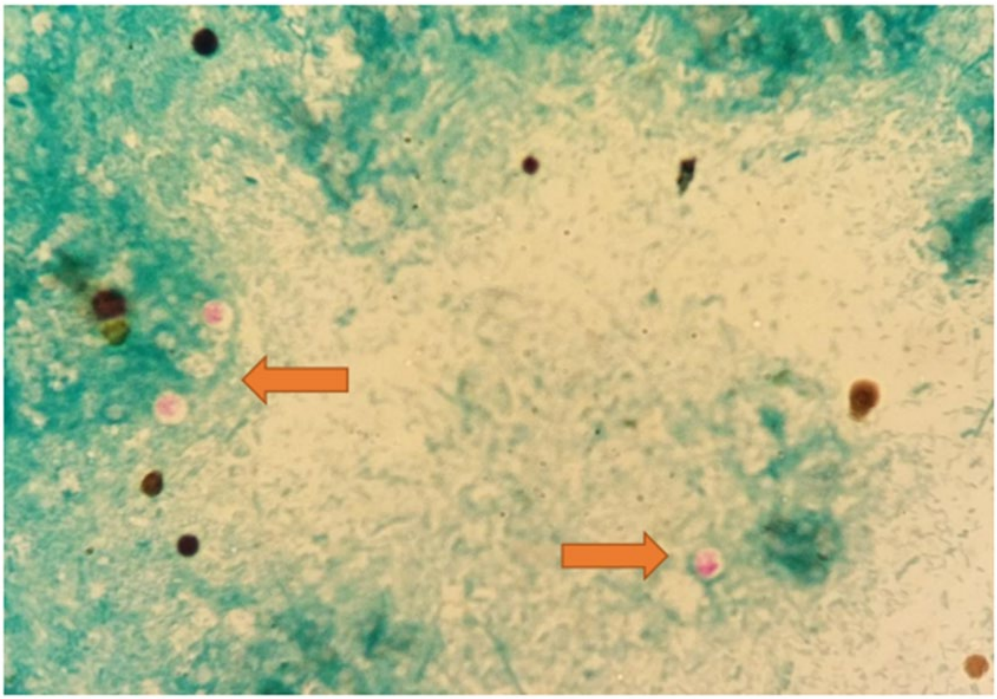

Figure 1. *Cryptosporidium* oocysts stained by Ziehl-Neelsen

Table 1. Microscopic evaluation of *Cryptosporidium* infection prevalence in examined animals

Characteristics		No (%)	Protozoa (*Cryptosporidium*)		*P value*
			Positive N(%)	Negative N(%)	
Animal	Cattle	63 (33.3%)	19(30.16%)	44(69.84%)	0.209
	Sheep	63 (33.3%)	13(20.63%)	50(79.37%)	
	Horse	63 (33.3%)	11(17.46%)	52(82.54%)	
Gender	Male	81 (42.9%)	27(33.33%)	54(66.67%)	0.003
	Female	108 (57.1%)	16(14.81%)	92(85.19%)	
Diarrhea	Positive	117 (61.9%)	38(32.47%)	79(67.53%)	0.000
	Negative	72 (38.1%)	5(6.94%)	67(93.06%)	

The findings of this study indicate a significant prevalence of *Cryptosporidium spp.* infection among domestic animals in Shiraz County, with an overall rate of 22.7% across 189 fecal samples. This prevalence highlights the potential risk of *Cryptosporidium spp.* as a zoonotic pathogen, particularly in agricultural settings where cattle, sheep, and horses are commonly raised. Cattle demonstrated the highest prevalence at 30.16%, suggesting a substantial role as reservoirs for *Cryptosporidium spp.* . The lower prevalence observed in sheep (20.63%) and horses (17.46%) suggests that while these species are involved in epidemiology of *Cryptosporidium spp.*, cattle likely contribute more

significantly to the transmission dynamics within the agricultural ecosystem.

The correlation between the presence of diarrhea and *Cryptosporidium spp.* infection is particularly noteworthy. The highest infection rate of 32.47% in fecal samples from animals exhibiting diarrheal symptoms emphasizes the importance of clinical signs in monitoring and managing *Cryptosporidium spp.* infections. This finding is consistent with existing literature that associates *Cryptosporidium spp.* infections with gastrointestinal distress in both ruminants and non-ruminants **(11, 12)**.

Our study is consistent with the findings from other regions regarding *Cryptosporidium spp.* prevalence in cattle. In a study examining *Cryptosporidium spp.* infection in cattle from Belgium, France, and the Netherlands, prevalence rates of 25.7%, 24.9%, and 20.8% were reported, respectively. These findings align closely with our study, which identified a prevalence rate of 30.16% in cattle in Shiraz County. While our prevalence rate is higher, the consistency in identification of *Cryptosporidium spp.* across different geographic regions underscores the widespread nature of this pathogen in cattle populations **(13)**. In a separate study conducted by Laatamna et al **(14)** to investigate *Cryptosporidium spp.* infection in 18 horses and 15 donkeys in Algeria, infection rates were reported as 2.3% and 1.6%, respectively **(14)**. Additionally, in a study conducted by Majewska et al **(15)** in Poland, which examined 159 sheep for *Cryptosporidium spp.* infection, an infection rate of 10.1% was reported. In our study, the prevalence of *Cryptosporidium spp.* infection in sheep and horses was found to be 20.63% and 17.46%, respectively. This comparatively higher prevalence in our study could be attributed, in part, to the larger sample size, which may have provided a more representative assessment of *Cryptosporidium spp.* infection rates in these livestock populations compared to studies with smaller cohorts. Furthermore, potential variations in environmental factors, animal management practices, and diagnostic methodologies between the study locations could also contribute to the observed differences in prevalence rates.

The severity of symptoms can vary based on the host's immunocompetence, with immunocompromised animals including those with concurrent health issues being more susceptible to severe manifestations **(16)**. The implications of these findings are significant for public health, as *Cryptosporidium spp.* can be transmitted to humans through direct contact with infected animals or contaminated environments **(17, 18)**.

Cryptosporidium spp. infection in humans can cause a range of symptoms; including diarrhea, abdominal cramps, nausea, and fever, and can be particularly severe in immunocompromised individuals. The severity and duration of the illness can vary significantly depending on the *Cryptosporidium spp.* and the host's immune status. Furthermore, persistent or recurrent *Cryptosporidium spp.* infections can lead to the chronic health problems **(19)**.

In livestock farmers, *Cryptosporidium spp.* infection can manifest similarly, causing diarrhea and other gastrointestinal issues, potentially impacting their productivity and well-being. Additionally, the presence of *Cryptosporidium spp.* in livestock can contribute to the contamination of food and water sources, thereby increasing the risk of human infection.

Further research is warranted to fully understand the prevalence and impact of *Cryptosporidium spp.* in livestock farming communities and the specific risks associated with different farming practices **(20)**. The presence of *Cryptosporidium spp.* in livestock raises concerns regarding zoonotic transmission, especially in regions where livestock and human populations intersect **(12, 21)**. The potential for environmental contamination, particularly through water sources, further necessitates rigorous monitoring and control measures. Given the high prevalence of *Cryptosporidium spp.* among symptomatic animals, it is crucial to implement comprehensive management strategies in livestock operations **(22, 23)**. This includes regular screening for *Cryptosporidium spp.*, improved sanitation practices, and education of farmers regarding the risks associated with infected animals **(24)**. Additionally, further research is needed to elucidate the specific *Cryptosporidium spp.* present in these populations and their potential zoonotic implications.

5. Conclusion

In conclusion, the study underscores the need for ongoing surveillance of *Cryptosporidium spp.* infections in domestic animals and the significance of addressing gastrointestinal issues in livestock to mitigate the risk of transmission to humans. These findings contribute to understanding *Cryptosporidium spp.* epidemiology and highlight the importance of veterinary and public health collaboration in managing infectious diseases in livestock.

6. Declarations

6.1 Acknowledgment

We would like to extend our sincere thanks to Mr Amir Alavi Mutabi, the head of the Parasitology Laboratory at the Faculty of Veterinary Medicine, Shiraz University, for his valuable collaboration in collection of animal samples for this project.

6.2 Ethical Considerations

The present study was conducted with the approval of the Ethical Committee of Shiraz University of Medical Sciences in Shiraz, Iran, under the ethical code IR.SUMS.AEC.1403.064. All ethical guidelines and principles were followed throughout the study.

6.3 Authors' Contributions

Imam Saleh Hassan: Data collection, study design. Kambiz Karimi: Drafting of the manuscript, data analysis. Asma Mousivand: Sample collection, manuscript review. Hossein Khosrow Panah: Assistance with data analysis. Mohammad Hossein Motazedian: Study design, supervision of the research, final review of the manuscript.

6.4 Conflict of Interests

The authors declare that they have no conflicts of interest.

6.5 Financial Support and Sponsorship

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6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

References

1. Mamedova S, Karanis P. Cryptosporidium spp. infections in livestock and wild animals in Azerbaijan territory. J Water Health. 2021;19(4): 545-62. [DOI:10.2166/wh.2021.050] [PMID]
2. Tang W, Akakulu W, Desai K. Pneumatosis intestinalis caused by Cryptosporidium colitis in a non-immunocompromised patient. IDCases. 2022;27:e01372. [PMID] [PMCID] [DOI:10.1016/j.idcr.2021.e01372]
3. Keener M, Waack A, Ranabothu M, Ranabothu A, Vattipally VR. Clinical and radiological features of Menetrier's disease: a case report and review of the literature. Cureus. 2023;15(9):e45537. [DOI:10.7759/cureus.45537]
4. Grout L, Marshall J, Hales S, Baker MG, French N. Dairy cattle density and temporal patterns of human campylobacteriosis and cryptosporidiosis in New Zealand. EcoHealth. 2022;19(2):273-89. [DOI:10.1007/s10393-022-01593-9] [PMID] [PMCID]
5. Jiang W, Roellig DM, Lebbad M, Beser J, Troell K, Guo Y, et al. Subtype distribution of zoonotic pathogen Cryptosporidium felis in humans and animals in several countries. Emerg Microbes Infect. 2020;9(1):2446-54. [PMID] [PMCID] [DOI:10.1080/22221751.2020.1840312]
6. Kabir MH Bin, Kato K. Comprehensive molecular epidemiology of Cryptosporidium species in Japan. Parasitol Int. 2024;100:102909. [DOI:10.1016/j.parint.2024.102909] [PMID]
7. Holsback L, Marquez ES, Silva MA da, Porto PP, Garcia JL, Martins FDC, et al. Molecular characterization of Cryptosporidium in ruminants and natural infection by Cryptosporidium andersoni in sheep from Paraná, Brazil. Rev Bras Parasitol Vet. 2023;32: e010023. [DOI:10.1590/s1984-29612023076] [PMID] [PMCID]
8. Burton AJ, Nydam DV, Dearen TK, Mitchell K, Bowman DD, Xiao L. Prevalence of Cryptosporidium and identification of the Cryptosporidium horse genotype in foals in New York State. Vet Parasitol. 2010;174(1-2): 139-44. [DOI:10.1016/j.vetpar.2010.08.019] [PMID]
9. Haghi MM, Khorshidvand Z, Khazaei S, Foroughi Parvar F, Sarmadian H, Barati N, et al. Cryptosporidium animal species in Iran: a systematic review and meta analysis. Trop Med Health. 2020;48:48. [PMID] [PMCID] [DOI:10.1186/s41182-020-00278-9]
10. Tahvildar Biderouni F, Salehi N. Detection of Cryptosporidium infection by modified Ziehl-Neelsen and PCR methods in children with diarrhea in pediatric hospitals in Tehran. Gastroenterol Hepatol Bed Bench. 2014;7(2): 125-31.
11. Díaz P, Navarro E, Prieto A, Pérez Creo A, Viña M, Díaz Cao JM, et al. Cryptosporidium species in post weaned and adult sheep and goats from NW Spain: public and animal health significance. Vet Parasitol. 2018;254:1-5. [DOI:10.1016/j.vetpar.2018.02.040] [PMID]
12. Firoozi Z, Sazmand A, Zahedi A, Astani A, Fattahi Bafghi A, Kiani Salmi N, et al. Prevalence and genotyping of Cryptosporidium in adult ruminants in central Iran. Parasit Vectors. 2019;

12:1-6. [DOI:10.1186/s13071-019-3759-2] [PMID] [PMCID]

13. Pinto P, Ribeiro CA, Hoque S, Hammouma O, Leruste H, Détriché S, et al. Cross border investigations of Cryptosporidium species in dairy cattle farms in western mainland Europe. Microorganisms. 2021;9(11):2394. [PMCID] [DOI:10.3390/microorganisms9112394] [PMID]
14. Wagnerová P, Sak B, Květoňová D, Xiao L, Rost M, McEvoy J, et al. Microsporidia and Cryptosporidium in horses and donkeys in Algeria: detection of a novel Cryptosporidium hominis subtype family (Ik) in a horse. Vet Parasitol. 2015;208(3-4):135-42. [DOI:10.1016/j.vetpar.2015.01.007] [PMID]
15. Majewska AC, Werner A, Sulima P, Luty T. Prevalence of Cryptosporidium in sheep and goats in west central Poland. Vet Parasitol. 2000; 89(4):269-75. [PMID] [DOI:10.1016/S0304-4017(00)00212-0]
16. Prabakaran M, Weible LJ, Champlain JD, Jiang RY, Biondi K, Weil AA, et al. The gut wrenching effects of cryptosporidiosis and giardiasis in children. Microorganisms. 2023;11(9):2323. [DOI:10.3390/microorganisms11092323] [PMID] [PMCID]
17. Becker DJ, Oloya J, Ezeamama AE. Household socioeconomic and demographic correlates of Cryptosporidium seropositivity in the United States. PLoS Negl Trop Dis. 2015;9(9):e0004008. [DOI:10.1371/journal.pntd.0004080] [PMID] [PMCID]
18. El Alfy E S, Nishikawa Y. Cryptosporidium species and cryptosporidiosis in Japan: literature review and animal transmission insights. J Vet Med Sci. 2020;82(8):1051-67. [PMID] [PMCID] [DOI:10.1292/jvms.20-0151]
19. Farthing MJG. Clinical aspects of human cryptosporidiosis. Contrib Microbiol. 2000;6:50-74. [DOI:10.1159/000060368] [PMID]
20. Santín M. Clinical and subclinical infections with Cryptosporidium in animals. N Z Vet J. 2013; 61(1):1-10. [DOI:10.1080/00480169.2012.731681] [PMID]
21. Robertson LJ, Johansen ØH, Kifleyohannes T, Efunshile AM, Terefe G. Cryptosporidium infections in Africa-importance of zoonotic transmission: a review. Front Vet Sci. 2020;7: 575881. [DOI:10.3389/fvets.2020.575881] [PMID] [PMCID]
22. Solarczyk P, Wojtkowiak Giera A, Heddergott M. Migrating Anatidae as sources of environmental contamination with zoonotic Giardia, Cryptosporidium, Cyclospora and microsporidia. Pathogens. 2023;12(3):487. [PMID] [PMCID] [DOI:10.3390/pathogens12030487]
23. Tram NT, Phuc PD, Phi NH, Trang LT, Nga TT, Ha HTT, et al. Cryptosporidium and Giardia in biogas wastewater: livestock manure management and hygiene aspects. Pathogens. 2022;11(2):174. [DOI:10.3390/pathogens11020174] [PMID] [PMCID]
24. Ogendo A, Obonyo M, Wasswa P, Bitek A, Mbugua A, Thumbi SM. Cryptosporidium infection in calves and the environment in Asembo, Western Kenya: 2015. Pan Afr Med J. 2017;28(Suppl 1):9. [PMID] [PMCID] [DOI:10.11604/pamj.supp.2017.28.1.9313]