

Cryptosporidiosis as a Persistent Threat to Livestock Health: An Epidemiological Study in Sheep and Cattle in Wasit Province, Iraq

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Abstract

Cryptosporidium parvum , a globally distributed coccidian protozoan, is a zoonotic pathogen that infects both humans and animals. This study was conducted to determine the incidence of *Cryptosporidium parvum* in sheep and cattle in Wasit Province of Iraq and its correlation with epidemiological parameters including age and sex. A total of 150 fecal samples were collected, including 75 samples from sheep and 75 samples from cattle, which were taken from different areas districts within Wasit province from January 2026 to May 2026. The samples were then examined using "Modified Ziehl-Neelsen stain" to determine the presence of *Cryptosporidium* oocysts in the samples. The results showed that the overall prevalence of *C. parvum* in cattle and sheep was 64/150 (42.67%), with 30/75 (40%) in cattle and 34/75 (45.33%) in sheep. The results also showed that the highest infection rate was recorded in animals under 6 months old, reaching 18/25 (72%) in cattle and 20/25 (80%) in sheep, while the infection rate gradually decreased with increasing age. Regarding sex, the infection rate in male cattle was 16/37 (43.2%) compared to 14/38 (36.8%) in females, while the infection rate in male sheep was 18/37 (48.6%) compared to 16/38 (42.1%) in females. The results indicate a high infection rate of *C. parvum* in both cattle and sheep, with a significantly higher infection rate observed among younger animals. However, no significant differences were observed between males and females in the infection rate within Wasit Province.

Keywords: *C. parvum*, Cattle , Sheep, Modified Ziehl-Neelsen stain.

Introduction

Cryptosporidium parvum , a globally distributed coccidian protozoan, is commonly found in surface waters and is currently a primary source of waterborne epidemics in the United States [1,2]. A recommended updated taxonomy is provided in parenthesis, "domain: Eukarya, phylum: Apicomplexa, class: Sporozoasida (Gregarinomorphea), order: Eucoccidiorida (Cryptogregarida), family: Cryptosporidiidae, genus: *Cryptosporidium* , species: *C. parvum*" [3] . *Cryptosporidium parvum* is "a

zoonotic pathogen composed of genetically distinct but morphologically identical genotypes" [4] . *Cryptosporidium* is a deadly protozoan parasite that infects the gastrointestinal epithelium of both humans and animals. Cryptosporidiosis in farm animals causes severe economic losses, including deaths of young animals and impaired growth, increased labor involved and high cost of drugs [5] . *Cryptosporidium* infection rates vary depending on the host species and geographical location [6] . According to [7] , both *Cryptosporidium hominis* and *Cryptosporidium parvum* can be found in

people, with *C. parvum* being primarily associated with infections in ruminant livestock, including cattle, sheep, and goats. This highlights *C. parvum*'s zoonotic potential and its important role in the animal-human transmission cycle.

Cryptosporidium parvum has different transmission routes, including firstly fecal-oral transmission, which occurs by the excretion of oocysts from infected animals in their feces to contaminate the environment. After that, healthy animals will be infected by swallowing the oocysts in food or water or directly [8]. Secondly, direct contact transmission, which occurs between infected animals and healthy animals, which leads to the transmission of oocysts, especially in unsanitary and crowded conditions [9]. Thirdly, environmental contamination with oocysts, which have a high resistance to cold and humid conditions as well as commonly used disinfectants, which is why they were a cause of environmental contamination and infection [10]. Fourthly, transmission through water contaminated with oocysts, which includes water used for drinking and water used for cleaning, thus causing infection in animals [11].

Preventive strategies include regular cleaning of animal housing, bedding, and equipment, as well as providing clean water and feed sources [8]. Isolating diseased animals helps to prevent oocysts from spreading to healthy ones [11]. Proper environmental management, such as keeping bedding dry and disposing of garbage, can reduce oocyst persistence and transmission [9].

Cryptosporidiosis parasites are diagnosed using a variety of techniques, the most important of which is microscopic examination of stool samples using the "Modified Ziehl-Neelsen stain (acid-fast stain)" which detects oocysts of *Cryptosporidium* and is the most often used approach for diagnosing cryptosporidiosis due to its ease, low cost, and availability of the dye

[12,13].

Due to the increasing incidence of diarrhea in cattle and sheep, and the prevalence of the parasite *C. parvum* in these animals, this study was conducted to detect this parasite in Wasit Governorate. The study aimed to diagnose parasite infection using modified Ziehl-Neelsen staining, while also investigating the effect of epidemiological factors such as age and sex on infection rates, in order to identify the groups most susceptible to infection. Furthermore, the study aimed to provide epidemiological data that would contribute to disease control and prevention, as well as help improve hygiene and health management on farms, reduce environmental pollution, and minimize the risk of animal-to-human transmission.

Materials and method

Ethical approval

This study was conducted in accordance with animal care and use standards, and received approval from the Ethics Committee of the College of Veterinary Medicine/Wasit University (Reference: WU-CVM.CS-BD-6 dated 13/5/2026 in Wasit Province, Iraq).

Study design

For this study, 150 fecal samples were collected, including 75 samples from sheep and 75 samples from cattle, which were collected from various districts within Wasit Province from January 2026 to May 2026. The fecal samples were subjected to the formalin-ether sedimentation technique to concentrate the oocysts prior to staining.

Detection of *C. parvum* in fecal samples

To obtain a better dyeing result when using the Modified Ziehl-Neelsen (MZN) staining method. The concentration approach was applied as follows: 1 gm of feces was mixed with 7ml of formalin(10%), followed by 3ml of the ether. The solution was forcefully stirred following the addition of the ether.

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Sediments were distributed on slides of the microscope before being dyed with modified Zeil-Neelson staining [14]. After concentration, the stool samples were stained by using modified Zeil-Neelson procedures. The slides that were inspected using a light microscope at (40X and 100X) magnification .

Statistical Analysis

The data were statistically analyzed using IBM SPSS Statistics version 26. The chi-square (χ^2) test was used to determine the statistical significance of differences between groups based on animal type, age, and gender. A p-value ($P \leq 0.05$) was considered statistically significant [15].

Results and Discussion

Infection rate of *C. parvum* in sheep and cattle

In this study, which was conducted on two groups of animals, a group of sheep and a group of cattle, using "the Modified Ziehl-Neelsen staining technique" for fecal samples, The results showed that the overall incidence of *C. parvum* in cattle and sheep was 64/150 (42.67%), where it was 30/75 (40%) in cattle and 34/75 (45.33%) in sheep as shown in Table 1 . The results showed a high incidence of *C. parvum* in cattle and sheep, but there were no significant differences between the incidence of cattle and sheep in Wasit Province.

Table (1): Comparison of *C. parvum* infection in sheep and cattle

Groups	Samples No.	<i>C. parvum</i> positive	Prevalence(%)
cattle	75	30	40%
sheep	75	34	45.33%
Total	150	64	42.67%
$\chi^2 = 0.428, P = 0.513$ (NS)			

The results of the current study showed a high incidence of *C. parvum* infection in cattle and sheep as shown in Table1. This may be attributed to the parasite's biological characteristics and its direct life cycle, which does not require an intermediate host, thus contributing to the rapid transmission and persistence of the infection among animals , The parasite is primarily transmitted via the fecal-oral route through contaminated water and feed, increasing the likelihood of its spread within barns and herds [16]. The oocysts are infectious once excreted in feces and possess high resistance to various environmental conditions, allowing them to survive for extended periods in contaminated water, soil, and feed, thereby increasing the likelihood of infection within herds. Furthermore, infected animals can shed large numbers of oocysts into the environment, leading to increased parasitic

load and the continuation of the infection cycle [17] .The high infection rate may also be linked to the nature of the farming systems used, as group farming and high animal density contribute to increased opportunities for direct and indirect contact between animals. Furthermore, the accumulation of animal waste and its failure to be disposed of regularly leads to increased environmental pollution within barns , The parasite's ability to survive for extended periods in animal waste, which facilitates the continuation of infection and its transmission between animals, as well as its access to water and food sources [18] . Pathologically, the parasite resides on the intestinal epithelial cells, causing malabsorption disorders and damage to intestinal cells, leading to diarrhea and malabsorption. In addition, it possesses virulence factors that enable it to adhere to,

invade, and multiply within cells, thus promoting the persistence and spread of infection [16]. A study in Wasit Province found that *C. parvum* infection rates were 20% in cattle and 14.5% in sheep utilizing the Z.N.S method [19]. Furthermore, research found *Cryptosporidium felis* in 17.69% of diarrhoeic calves in Wasit Province [7]. These findings demonstrate the high incidence of *Cryptosporidium* spp. in livestock in Wasit Province, emphasizing the importance of effective control strategies to limit illnesses and economic losses. In Baghdad Province, a study found a 38% prevalence of *Cryptosporidium* spp. in cattle fecal samples [20]. The global prevalence of *Cryptosporidium* infection in sheep was about 18.9% during an analytical study conducted on these animals, with regional differences in the incidence rate, as the highest rate was in North America (29.8%),

followed by Africa (21.7%) and Europe (20.2%), while the lowest incidence rate of the parasite was 14.8% in Asia [21]. Another study reported that the global prevalence of *Cryptosporidium* infection in cattle was 25.5% and identified that the most common species is *C. parvum* [22].

Infection rate of *C. parvum* in cattle according to age The results showed that the highest infection rate with *C. parvum* parasite in cattle was recorded in calves under 6 months old, at 18/25 (72%), followed by the 6–12 month age group at 9/25 (36%). The lowest infection rate was recorded in cattle over one year old, at 3/25 (12%), as shown in Table 2. The results indicate that young animals were more susceptible to infection.

Table (2): Infection rate of *C. parvum* in cattle according to age

Age groups	Samples No.	<i>C. parvum</i> positive	Prevalence(%)
< 6 months	25	18	72 %
6-12 months	25	9	36 %
> year 1	25	3	12 %
Total	75	30	40 %
$\chi^2 = 19.091, P < 0.001$ (HS)			

The results of the current study, shown in Table 2, indicate that the highest incidence of *C. parvum* infection was recorded in calves under six months of age, while the infection rate decreased significantly with increasing age. This can be explained by the fact that young animals are less resistant to infection compared to older animals, giving them an important epidemiological role in perpetuating the infection cycle within herds. Furthermore, *Cryptosporidium* is a major cause of diarrhea in newborn calves due to its impact on animal health and growth, making this age group more susceptible to infection [11,23]. Pre-weaning calves are also the primary source of *C. parvum* within herds because they can shed large quantities of oocysts into the environment, increasing environmental contamination and

the likelihood of infection spreading to other animals. This contributes to the continuation of the parasite's life cycle and increases the infection rate in young animals [24]. The lower incidence in older age groups may be due to the development of some degree of resistance or immune response after initial exposure to the parasite, leading to a decrease in the severity or frequency of infection with advancing age [25]. As a result, the relationship between host susceptibility, environmental exposure levels, and the persistence of the infection cycle within the herd is reflected in differences in infection rates among age groups.

Infection rate of *C. parvum* in sheep according to age

The results showed that the highest infection rate with *C. parvum* parasite, in sheep was recorded in lambs under 6 months old, at 20/25 (80%), followed by the 6–12 month age group

at 10/25 (40%). The lowest infection rate was recorded in sheep over one year old, at 4/25 (16%), as shown in Table 3.

Table (3): Infection rate of *C. parvum* in sheep according to age

Age groups	Samples No.	<i>C. parvum</i> positive	Prevalence(%)
< 6 months	25	20	80 %
6-12 months	25	10	40 %
> year 1	25	4	%16
Total	75	34	45.33 %
$\chi^2 = 21.123$, $P < 0.001$ (HS)			

The results of the current study showed that lambs under 6 months of age had the highest incidence of *C. parvum* infection compared to older lambs as shown in Table 3, indicating that the younger age group is the most susceptible to infection among the studied samples. This may attributed to the fact that this age group is most affected by the parasite's life cycle and transmission within a contaminated environment, where exposure to oocysts shed in feces is more likely, especially in conditions of high animal density and poor sanitation. [26] indicated that *Cryptosporidium* is one of the most important causes of diarrhea in small ruminants, and [27] showed that young animals are more susceptible to infection than older animals. Similarly, [28] demonstrated that young lambs exhibit a high susceptibility to *Cryptosporidium*

infection. Accordingly, the high infection rates in young lambs reflect the important role of age as a key epidemiological factor in determining the severity of infection spread within flocks, in addition to the impact of environmental and management factors on the continuation of the parasite's cycle and the increased chances of its transmission between animals.

Infection rate of *C. parvum* in cattle according to sex

The results showed that the infection rate in male cattle was 16/37 (43.2%), while the infection rate in females was 14/38 (36.8%), whereas , in male sheep was 18/37 (48.6%), while the infection rate in females was 16/38 (42%), as shown in Table 4. A slight difference in infection rate between the sexes was observed.

Table (4): Infection rate of *C. parvum* in cattle according to sex

Groups	Samples No.	<i>C. parvum</i> positive	Prevalence(%)
Male	37	16	43.2 %
Female	38	14	36.8 %
Total	75	30	40 %
$\chi^2 = 0.316$, $P = 0.574$ (NS)			

The results of the current study showed a slight difference in the incidence of *C. parvum* infection between male and female cattle as shown in Table 4. However, this difference was not statistically significant, suggesting that sex is not a major factor in

infection. This is explained by the fact that parasite transmission depends more on the level of environmental exposure and the severity of contamination within barns than on physiological parameters related to sex. [29] indicated that environmental conditions

play a greater role than sex in *Cryptosporidium* transmission, confirming that the animal's surrounding environment is the most important factor in the continuation and spread of the infection cycle within herds. [30] also reported no significant differences between males and females, indicating similar infection rates between the sexes and no influence from sex. Similarly, [31] stated that there is no significant correlation between sex and *Cryptosporidium* infection. Accordingly, it is clear that the slight variation in infection rates between males and females is due more to environmental and management factors than

to being related to sex, such as the level of pollution, breeding density, and health management conditions within barns, which are the main determinants in the dynamics of the spread of the parasite among cows.

Infection rate of *C. parvum* in sheep according to sex

The results showed that the infection rate in male sheep was 18/37 (48.6%), while the infection rate in females was 16/38 (42%), as shown in Table 5 . A slight difference in infection rate between the sexes was observed.

Table (5): Infection rate of *C. parvum* in sheep according to sex

Groups	Samples No.	<i>C. parvum</i> positive	Prevalence(%)
Male	37	18	48.6 %
Female	38	16	42.1 %
Total	75	34	45.33 %
$\chi^2 = 0.312, P = 0.577$ (NS)			

The results of the current study showed a slight difference in the incidence of *C. parvum* infection between male and female sheep Table 5 . However, this difference was not statistically significant, indicating that sex is not a major factor in infection. This is explained by the fact that transmission of the infection depends primarily on the level of environmental contamination and the routes of exposure to the parasite, rather than on biological differences between the sexes.

In this context [32] explained that transmission of the parasite's oocysts occurs mainly through contaminated food and water, demonstrating that a contaminated environment is the most important factor in the occurrence and persistence of infection. [30], also confirmed that sex is not a significant risk factor for *Cryptosporidium* infection, indicating the similarity in infection rates between males and females and their lack of direct influence by sex.

Therefore, it is clear that the slight differences in infection rates between males and females are due to differences in environmental exposure levels and rearing conditions within the pens, such as hygiene, animal density, and the availability of water and feed sources, and not to a direct effect of sex. This confirms that environmental factors remain the decisive factor in the dynamics of the spread of the *C. parvum* parasite among sheep.

Several variables influence *Cryptosporidium* infection in animals, including the following: young animals, particularly newborns, are more susceptible due to their underdeveloped immune systems, but older animals develop partial immunity, which lessens the severity of infection with time [8]. Co-infection with other intestinal infections, such as rotavirus or coronavirus, can aggravate the sickness and affect transmission [9].

Conclusion

This study discovered the prevalence of *C. parvum* in sheep and cattle in Wasit Governorate, Iraq, with an overall infection rate of 42.67%, including 40% in cattle and 45.33% in sheep. The data also revealed a significant infection rate in young animals < 6-months of age, with modest differences between males and females. These findings show that *C. parvum* is definitely prevalent among ruminants in Wasit Governorate, which could have a negative impact on livestock

health and productivity. The report emphasizes the necessity of improving agricultural sanitation, conducting frequent inspections, and establishing health management programs to minimize the spread of infection. Future research should focus on seasonal variations in infection rates and potential risk factors contributing to its spread.

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