Research Article

Conventional Detection of *Cryptosporidium* spp. in Sheep and Their Owners in Wasit Province, Iraq

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Abstract | Cryptosporidiosis is an intestinal parasitic infection of significant veterinary and public health importance, particularly in rural communities where close contact exists between livestock and humans. Conventional microscopy remains a practical and widely used method for the detection of *Cryptosporidium* oocysts in fecal samples in many local laboratories. This cross-sectional study was conducted in five areas of Wasit Province, Iraq. A total of 150 samples were collected, including 75 human stool samples and 75 sheep fecal samples. All samples were examined using modified Ziehl–Neelsen staining. The results were analyzed according to host, sex, age group, and region using Pearson's chi-square test. Out of 150 examined samples, 48 were positive, yielding an overall prevalence of 32.00%. The prevalence was significantly higher in sheep compared to humans (41.33% vs. 22.67%; χ^2 = 5.18, P = 0.023). In both humans and sheep, no significant associations were observed between infection rate and region, sex, or age group (P > 0.05). Microscopically, positive samples exhibited acid-fast, round to ovoid structures consistent with *Cryptosporidium* oocysts. In conclusion, conventional microscopy confirmed the presence of *Cryptosporidium* infection in both sheep and their owners in Wasit Province. The higher prevalence in sheep suggests their potential role in local transmission, while the distribution pattern indicates shared environmental exposure at the animal–human interface.

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Introduction

Cryptosporidiosis is an intestinal disease caused by protozoa of the genus *Cryptosporidium*. The parasite infects a wide range of hosts, including humans and domestic animals, and is mainly transmitted by the fecal–oral route through contaminated water, food, fomites, or direct contact with infected hosts. Because the oocysts are shed in an infective state and can remain viable in the environment, *Cryptosporidium* continues to be important in both veterinary and public health contexts (Fayer, 2010; Checkley *et al.*, 2015; Helmy and Hafez, 2022).

Sheep play a meaningful role in the epidemiology of cryptosporidiosis. In small ruminants, infection

may be linked to diarrhea, poor growth, and ongoing contamination of the surrounding environment. In rural settings, close contact between people and infected animals, or with contaminated housing and soil, may increase the chance of zoonotic exposure (Paraud and Chartier, 2012; Santin, 2013; Guo *et al.*, 2021).

In Iraq, sheep husbandry is common in rural and peri-rural communities, and concern about *Cryptosporidium* at the animal–human interface has grown in recent years. Even so, paired local information on infection in sheep and their owners remains limited, particularly in Wasit Province (Alali *et al.*, 2021; Abdullah and Mohammed, 2024).

Conventional microscopic techniques are still widely used in local laboratories because they are inexpensive, accessible, and relatively easy to apply. Among these methods, modified Ziehl–Neelsen staining remains useful for demonstrating acid-fast oocysts in fecal smears, although interpretation can be influenced by fecal debris, staining quality, and parasite load (Ahmed and Karanis, 2018; Abdullah and Mohammed, 2024). Accordingly, the present study aimed to investigate the conventional microscopic detection of *Cryptosporidium* spp. in sheep and their human owners in Wasit Province, Iraq, with statistical analysis based on host, sex, age, and region.

Materials and Methods

Study design and area

This study was designed as a cross-sectional parasitological investigation conducted in Wasit Province, Iraq. Sampling was performed in five areas Kut, Al-Hai, Al-Numaniyah, Al-Suwaira, and Badra between October and December 2025. These locations were selected because sheep rearing is common and routine contact between animals and their owners takes place under everyday field conditions.

Study population and samples

The full dataset consisted of 150 fecal specimens, including 75 human stool samples collected from sheep owners and 75 fecal samples collected from sheep. In the microscopy-based conventional phase, all 150 samples were available for analysis, comprising 75 human samples and 75 sheep samples. This microscopy dataset also retained information on region, sex, and age, which allowed subgroup analysis within the traditional diagnostic component.

Sample collection and handling

Fresh human and sheep fecal samples were collected in clean sterile plastic containers. Approximately 10 g of material was placed in each container and labeled using the available field data. The samples were transported under cooled conditions at approximately 4 °C to the parasitology laboratory of the College of Veterinary Medicine, Al-Qasim Green University, where material intended for conventional examination was processed after arrival.

Conventional parasitological examination

The conventional diagnostic phase included direct smear examination, concentration-based examination, and modified Ziehl–Neelsen staining. These methods were chosen because they remain among the most commonly used traditional approaches for identifying *Cryptosporidium* oocysts in fecal specimens in routine parasitology laboratories (Ahmed and Karanis, 2018; Abdullah and Mohammed, 2024). Direct examination was carried out by emulsifying a small amount of fecal material on a clean glass slide to prepare a thin smear for microscopic evaluation. Concentration-based procedures were also used to improve the chance of detecting oocysts in samples with a low parasite burden.

Flotation technique

In addition to direct smear examination, flotation techniques were employed to improve the recovery of *Cryptosporidium* oocysts from fecal samples. Approximately 2–3 g of fecal material was mixed with the flotation solution and thoroughly homogenized. The suspension was filtered through gauze to remove coarse debris and transferred into a centrifuge tube.

Flotation techniques were performed following the methods described by Zajac and Conboy (2012) and Dryden *et al.* (2005) using Sheather's sugar solution. The tube was filled to form a convex meniscus, and a clean coverslip was placed on top. After allowing the preparation to stand for 10–15 minutes, the coverslip was carefully removed and placed onto a glass slide for examination under a light microscope.

Oocysts were identified based on their characteristic size, shape, and refractile appearance. The flotation technique was applied as a concentration method to enhance detection, particularly in samples with low

parasitic load (Soulsby, 1982).

Modified ziehl–neelsen staining

Modified Ziehl–Neelsen staining was selected because *Cryptosporidium* oocysts are acid-fast and typically appear as pink to red round or ovoid bodies against a blue background. The staining procedure followed standard methods described for fecal diagnosis of cryptosporidiosis (Ahmed and Karanis, 2018; Abdullah and Mohammed, 2024). Briefly, a drop of fecal suspension was spread on a clean slide, air-dried, and fixed in concentrated methanol. The smear was then stained with carbol fuchsin for about 5 minutes, rinsed with water, briefly decolorized in acid solution for approximately 15 seconds, and counterstained with methylene blue for about 5 minutes. The stained smears were examined under oil immersion. A smear was considered positive when acid-fast bodies morphologically compatible with *Cryptosporidium* oocysts were seen.

Statistical analysis

Microscopy findings were evaluated by host type, sex, age group, and region. Human samples were grouped by age as <9, 10–19, 20–39, 40–59, and >60 years, while sheep samples were grouped as <1 year, 1–<3 years, and ≥3 years. Frequencies and percentages were calculated, and Pearson's chi-square test was used to assess associations between categorical variables. A P value of less than 0.05 was considered statistically significant.

Results

Overall microscopic detection according to the host

Microscopy revealed *Cryptosporidium* oocysts in 48 of the 150 examined smears, corresponding to an overall positivity rate of 32.00%. The proportion of positive samples was higher in sheep than in humans. Among the 75 sheep smears, 31 were positive (41.33%), whereas 17 of 75 human smears were positive (22.67%). This difference was statistically significant (χ^2 = 5.18, P = 0.023), indicating a greater rate of microscopic detection in sheep within the conventionally examined dataset (Table 1).

Human microscopy results according to region

Among the 75 human samples included in the microscopy dataset, positivity varied across the five study areas. Al-Suwaira showed the highest crude positivity rate at 33.33%, whereas Kut showed the lowest at 13.33%. No significant association was detected between human positivity and region (χ^2 = 1.98, P = 0.740, Table 2).

Table 1: *Microscopic detection of Cryptosporidium spp. according to the host.*

Host	Examined	Positive	Negative	Positivity (%)
Humans	75	17	58	22.67
Sheep	75	31	44	41.33*
Total	150	48	102	32.00

** Statistically higher than human (P=0.023).*

Table 2: *Human microscopy results according to region.*

Region	Examined	Positive	Negative	Positivity (%)
Kut	15	2	13	13.33
Al-Hai	15	3	12	20.00
Al-Numaniyah	15	4	11	26.67
Al-Suwaira	15	5	10	33.33
Badra	15	3	12	20.00
Total	75	17	58	22.67
P-value				0.740

Human microscopy results according to sex

In the human group, positivity was higher in males than in females. Of the 43 male samples, 12 were positive (27.91%), compared with 5 of 32 female samples (15.63%). This difference was not statistically significant (χ^2 = 0.96, P = 0.328, Table 3).

Table 3: *Human microscopy results according to sex.*

Sex	Examined	Positive	Negative	Positivity (%)
Male	43	12	31	27.91
Female	32	5	27	15.63
Total	75	17	58	22.67
P-value				0.328

Human microscopy results according to age group

Among humans, the highest crude positivity rate was recorded in the 20–39-year group, where 8 of 27 samples were positive (29.63%). Positivity was 22.22% in children younger than 9 years, 25.00% in the 10–19-year group, and 0.00% in both the 40–59-year and >60-year groups. No significant association was found between age group and positivity (χ^2 = 4.06, P = 0.398, Table 4).

Sheep microscopy results according to region

Within the sheep subset, positivity ranged from

33.33% in Kut and Badra to 53.33% in Al-Suwaira (Table 5). Despite this variation, the regional differences were not statistically significant (χ^2 = 1.87, P = 0.760).

Table 4: *Human microscopy results according to age group.*

Age group (years)	Examined	Positive	Negative	Positivity (%)
<9	9	2	7	22.22
10–19	28	7	21	25.00
20–39	27	8	19	29.63
40–59	7	0	7	0.00
>60	4	0	4	0.00
Total	75	17	58	22.67
P-value				0.398

Table 5: *Sheep microscopy results according to region.*

Region	Examined	Positive	Negative	Positivity (%)
Kut	15	5	10	33.33
Al-Hai	15	6	9	40.00
Al-Numaniyah	15	7	8	46.67
Al-Suwaira	15	8	7	53.33
Badra	15	5	10	33.33
Total	75	31	44	41.33
P-value				0.760

Sheep microscopy results according to sex

Female sheep had a slightly higher positivity rate than male sheep. Of the 44 females examined, 19 were positive (43.18%), whereas 12 of 31 males were positive (38.71%). This difference did not reach statistical significance (χ^2 = 0.02, P = 0.881, Table 6).

Table 6: *Sheep microscopy results according to sex.*

Sex	Examined	Positive	Negative	Positivity (%)
Male	31	12	19	38.71
Female	44	19	25	43.18
Total	75	31	44	41.33
P-value				0.881

Sheep microscopy results according to age group

In sheep, microscopy positivity was 100.00% in animals younger than 1 year, 45.00% in those aged 1–<3 years, and 38.89% in animals aged ≥3 years (Table 7). No significant association was observed between age group and positivity (χ^2 = 1.66, P = 0.435).

Table 7: *Sheep microscopy results according to age group.*

Age group	Examined	Positive	Negative	Positivity (%)
<1 year	1	1	0	100.00
1–<3 years	20	9	11	45.00
≥3 years	54	21	33	38.89
Total	75	31	44	41.33
P-value				0.435

Microscopic appearance

Modified Ziehl–Neelsen-stained smears showed round to ovoid acid-fast bodies that appeared pink to red against a pale blue background, a morphology consistent with *Cryptosporidium* oocysts. The representative image retained from the study material supports the microscopic diagnosis used in the conventional phase (Figures 1-2).

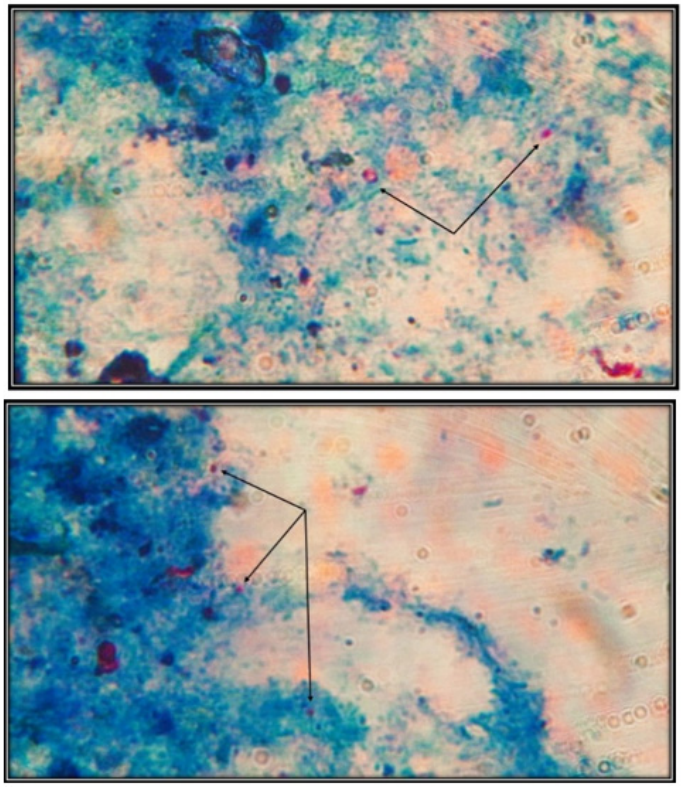

Figure 1: *Representative modified Ziehl–Neelsen-stained fecal smears showing acid-fast bodies morphologically compatible with Cryptosporidium oocysts in Sheep (arrows).*

Discussion

This study provided microscopic evidence of *Cryptosporidium* infection in both sheep and their human owners in Wasit Province. The most notable finding was the significantly higher positivity rate detected in sheep. From an epidemiological perspective,

this pattern is plausible because sheep can sustain fecal contamination within flock environments and may contribute to repeated exposure at the animal–human interface (Paraud and Chartier, 2012; Santin, 2013; Guo *et al*., 2021).

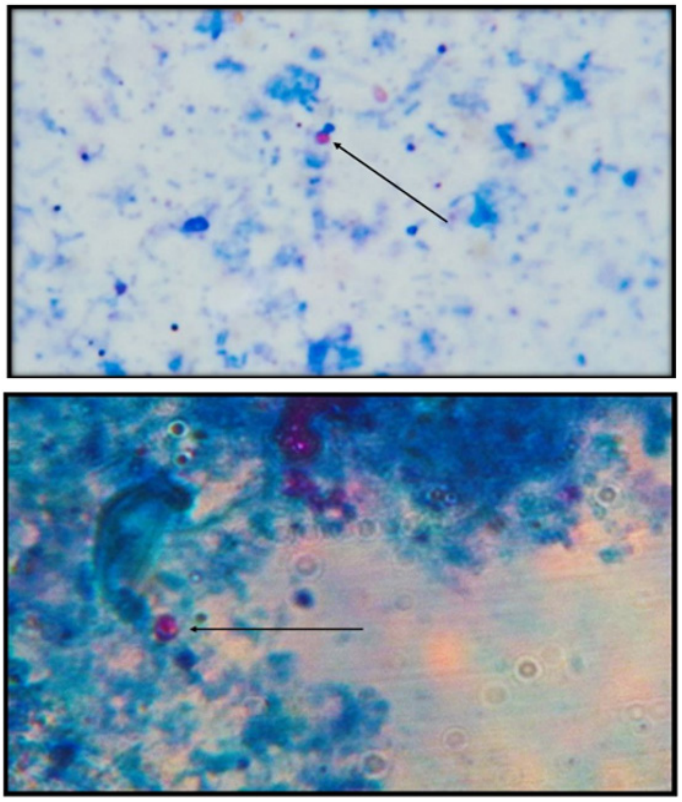

Figure 2: *Representative modified Ziehl–Neelsen-stained fecal smears showing acid-fast bodies morphologically compatible with Cryptosporidium oocysts in Human (arrows).*

The higher positivity observed in sheep may reflect ongoing contamination of bedding, feed, water sources, and pen surfaces under field conditions. By contrast, human infection is influenced not only by contact with animals but also by hygiene practices, manure handling, and exposure to contaminated water or shared surroundings (Hunter and Thompson, 2005; Ali *et al*., 2024).

No significant association was found between positivity and sex, age, or region in either humans or sheep. This may suggest that exposure in the sampled setting was fairly widespread rather than concentrated in one subgroup. In humans, the highest crude positivity was observed in Al-Suwaira and in adults aged 20–39 years, while in sheep, the highest regional positivity was observed in Al-Suwaira and the highest age-specific positivity was recorded in animals younger than 1 year; however, these subgroup differences were not statistically significant. Similar variability across age, sex, and locality has been described in reviews of animal and human cryptosporidiosis, especially where management systems and environmental conditions differ between studies (Alali *et al*., 2021; Chen *et al*., 2022).

The absence of significant regional variation further suggests that exposure to *Cryptosporidium* was not confined to a single district of Wasit Province. Instead, the parasite appears to be distributed across the surveyed areas, which is in line with the broad environmental nature of cryptosporidial transmission in livestock-associated settings (Ali *et al*., 2024; Ryan *et al*., 2021).

The modified Ziehl–Neelsen technique was useful for documenting morphologically compatible oocysts and remains relevant for routine parasitology laboratories in Iraq because it is inexpensive and simple to perform. Even so, its diagnostic performance can be influenced by staining quality, parasite burden, and observer experience, and the method alone cannot determine the species involved (Ahmed and Karanis, 2018; Smith and Nichols, 2010).

A strength of the study is that it links animal and human parasitology within the same province using material collected under field conditions. In addition, the microscopy analysis in the present version was based on the full conventional dataset of 150 samples rather than a reduced subset, which improves the stability of the descriptive estimates and subgroup comparisons. Even so, some subgroup cells remained relatively small and this should be taken into account when interpreting the chi-square results, particularly for the sheep age analysis.

Taken together, the findings reinforce the continuing veterinary and public health importance of cryptosporidiosis in Iraq. Although microscopy on its own cannot demonstrate direct zoonotic transmission, detecting *Cryptosporidium* oocysts in both sheep and their owners is consistent with shared environmental exposure and highlights the need for practical hygiene measures in rural production systems (Hunter and Thompson, 2005; Alali *et al*., 2021; Ali *et al*., 2024).

Conclusion

Traditional microscopic examination demonstrated

the presence of *Cryptosporidium* oocysts in both sheep and their human owners in Wasit Province, Iraq. Sheep showed a significantly higher positivity rate than humans, suggesting that they may play an important role in environmental contamination in the local setting. No significant associations were observed with sex, age, or region in either host group. Although these findings do not establish direct zoonotic transmission, they support the likelihood of shared exposure at the animal–human interface and underline the need for continued surveillance and practical hygienic control measures in rural communities.

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Novelty Statement

This study represents one of the limited epidemiological investigations using traditional microscopic techniques for the diagnosis of Cryptosporidium spp. in Sheep and Their Owners in Wasit Province, Iraq. The study provides updated data regarding the prevalence of infection according to sex, age groups, and geographical distribution.

Author's Contribution

Ali Ehsan Jasim performed sample collection, laboratory examination, statistical analysis, and manuscript preparation. Qasim Jawad Amer supervised the study, reviewed the manuscript, and contributed to data interpretation.

Generative AI and AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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