Microscopic detection of Cryptosporidiosis in cattle, Human and Environmental samples in Basrah province

Raghad Shreif Ibrahim[1], Muna M. Jori[2], Murtakab Y. Obeed[3]

1- Basra Univ., Medicine College, Microbiology Dept.,

2- Basra Univ., Vet. College, Parasitology Dept.

3-Basra Univ., Vet. College, Microbiology Dept.

Abstract

Cryptosporidium spp. is a pathogenic protozoan parasite of considerable public health as well as veterinary importance, particularly in developing countries. The current investigation was designed to determine the frequency of *Cryptosporidium* infection in human, cattle, and environmental samples collected from different regions of Basrah province, Iraq. amount of 400 stool specimens from humans and cattle were examined in addition to water and soil samples using conventional parasitological techniques. The general frequency of *Cryptosporidium* infection was 32.33%, with a significantly higher infection rate recorded in cattle (36%) compared to humans (28.66%). In cattle, infection rates were significantly associated with sex, age, and geographical region, whereas in humans, age and region showed significant associations, while no significant difference was observed between sexes. Environmental examination revealed the existence of *Cryptosporidium* oocysts in Water and soil specimens, although the prevalence was relatively low and showed no significant regional variation. These findings confirm the epidemiological importance of cattle as a potential reservoir for *Cryptosporidium* spp. and showcase the possible function of environmental contamination in maintaining The transmission period.

Keywords: Cryptosporidium spp., Cattle, Human, Soil, Water

Introduction

Cryptosporidium is a obligate The parasitic intracellular protozoa that causes cryptosporidiosis, a zoonotic illness affecting humans and animals globally[1] . The parasite forms environmentally resistant oocysts capable of surviving for extended periods, facilitating transmission via contaminated water, food, and direct contact[2]. Even minimal exposure can lead to clinical illness, highlighting its high infectivity and public health relevance[3]. In livestock, especially neonatal calves, *Cryptosporidium parvum* is a major contributor to enteric illnesses and diarrhea, resulting in dehydration, growth retardation, and economic losses in cattle production[4]. Infected animals serve as reservoirs for zoonotic transmission, posing risks to farmers and rural communities[5]. Environmental contamination with *Cryptosporidium* oocysts is well-documented in water bodies and agricultural soils, particularly in areas with intensive livestock activity and poor sanitation[6]. These oocysts resist conventional water treatment and persist in moist environments, enabling transmission to humans and animals through ingestion or contact[7]. Calves contribute to environmental shedding, while human exposure is often linked to untreated water and contaminated soil[8]. In Iraq, infections have been reported in humans and cattle across provinces such as Basrah,

ISSN 2072-3857

Karbala, and Duhok[9]. Molecular studies identified zoonotic species, mainly *C. parvum* and *C. hominis*, with genetic diversity among isolates[10, 11]. Diagnosis typically involves microscopic examination using the modified Ziehl–Neelsen acid-fast stain[12]. However, ELISA and PCR techniques are increasingly adopted for their sensitivity and ability to differentiate species and genotype[1] . Given the parasite's zoonotic nature and environmental persistence, integrated surveillance and molecular characterization are vital for effective control. This study aims to assess The occurrence of *Cryptosporidium* spp. in cattle and humans in southern Iraq and compare findings with global patterns[13].

Material and Methods

study region and collection of samples: This research was conducted to identify *Cryptosporidium* spp. in fecal, water, and soil samples collected from various regions of Basrah Province. The collection samples were between March 2024 and March 2025. All procedures followed conventional parasitological techniques, relying on the Centers for Disease Control and Prevention's modified Ziehl-Neelsen staining technique (CDC DPDx, 2023). Fecal samples collection from various region (South, North, East and West) of basrah Province. Human and bovine fecal samples were collected from outpatient clinics and rural farms, respectively. Approximately 5–10 g of fresh stool was placed in sterile containers, labeled with source and location data, and transported under refrigeration at 4°C to the laboratory within four hours. Each sample was emulsified in phosphate-buffered saline (PBS), filtered through sterile gauze, and centrifuged for ten minutes at 3000 rpm.. The sediment was retained for smear preparation. Water samples (1 liter each) were obtained from rivers, irrigation canals, drinking water tanks, and wastewater outlets using sterile polyethylene bottles. Samples were filtered through 1.2 µm membrane filters; the retained material was eluted with PBS and concentrated by centrifugation at 3000 rpm for 15 minutes[14]. Soil samples (approximately 100 g) were collected from agricultural and livestock zones at a depth of 5–10 cm using sterile spatulas. Ten grams of each sample were suspended in 50 mL PBS, shaken vigorously, allowed to settle, and the supernatant was centrifuged to obtain sediment. Smears were prepared from the sediment of each sample type, air-dried, and heat-fixed. Slides were stained with a modified Ziehl-Neelsen method: carbol fuchsin staining for ten minutes, acid-alcohol decolorization for one minute, and methylene blue counterstaining for one minute. Microscopic examination was performed under oil immersion at 1000× magnification. *Cryptosporidium* oocysts were identified as bright red to pink spherical or ovoid bodies measuring 4–6 µm against a blue background (CDC DPDx, 2023). All reagents were freshly prepared, and negative control slides were included in each staining batch. Duplicate readings were performed by independent microscopists to ensure accuracy and reproducibility.

Laboratory Examination of Fecal Samples:

Macroscopic examination: were analyzed macroscopically to evaluate color and consistency. abnormalities as well as the existence of blood, mucus, or other peculiar elements[15].

Microscopic examination: This study aimed to detect *Cryptosporidium* spp. in fecal, water, and soil samples collected from different regions of Basrah Province using conventional parasitological techniques. Direct wet smears were

ISSN 2072-3857

prepared from stool samples diluted with water and examined microscopically under increasing magnifications.

Identification was primarily performed using the modified Ziehl–Neelsen (MZN) staining technique. Smears were made using sample sediments, fixed with methanol, decolorized after ten minutes of staining with carbol fuchsin with ethanol for one minute, counterstained, and examined at 400× and 1000× magnification as described by Henriksen and Pohlenz (2021) and recommended by the CDC (CDC DPDx, 2023).

The flotation method was also used for stool examination. Diluted stool samples were mixed with Lugol's iodine and flotation solution until a convex meniscus formed, covered with a coverslip, and left for 30 minutes before microscopic examination to detect oocysts, cysts, and trophozoites[16]. Human and bovine fecal samples were collected in sterile containers, transported at 4 °C, processed with phosphate-buffered saline (PBS), filtered, and centrifuged to obtain sediments. Water samples (1 L) were filtered through membrane filters and concentrated by centrifugation[14]. Soil samples were collected from agricultural and livestock areas, suspended in PBS, and centrifuged to recover sediments[17]. *Cryptosporidium* oocysts were identified as red to pink spherical or ovoid bodies measuring 4–6 µm against a blue background. Negative controls and duplicate slide examinations were performed to ensure accuracy and reproducibility.

Results and Discussion

A *Cryptosporidium* spp. is observed microscopically in the form of oocysts. The oocysts are small, round to ovoid in shape, with a thick, double-layered wall.

The oocysts measure approximately 4–6 µm in diameter, making them among the smallest intestinal protozoan parasites detected in stool samples (Figure 1).

With routine stains, oocysts may be difficult to identify. However, when stained using the oocysts are visible with the Modified Ziehl-Neelsen (acid-fast) stain. pink to bright red, while the background stains blue or green. Some oocysts may appear faintly stained or unstained (ghost cells).

Total of 300 fecal samples from humans and cattle (fig. 2) were examined, of which 97 (32.33%) were positive for *Cryptosporidium* spp. As seen in table (1) The pathogen was detected in 43 out of 150 human samples (28.66%) and 54 out of 150 cattle samples (36%), with a statistically significant difference between the two hosts ($p \leq 0.05$). Among cattle, male animals showed a significantly higher infection rate (56.66%) compared to females (30.83%) (p is less than 0.05) as recorded in the Table (2), whereas the table (3) appeared no significant difference observed between male and female human samples (p is less than 0.05). Significant variation in infection rates was recorded among different regions of Basrah for both humans and cattle (p is less than 0.05). With regard to age (table 9), infection rates were significantly higher in younger age groups among humans and cattle compared to older groups ($p \leq 0.05$). Environmental examination revealed present of *Cryptosporidium* oocysts in 8% of water samples (fig.3) and 6% of samples of soil (fig.4), with no statistically significant difference among regions (p is less than 0.05) as shown tables 8 and 9.

ISSN 2072-3857

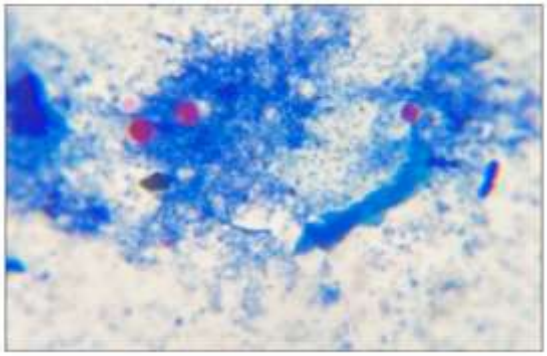

Figure (1): Modified Ziehl-Neelsen in human fecal samples (100X). *cryptosporidium* sp.

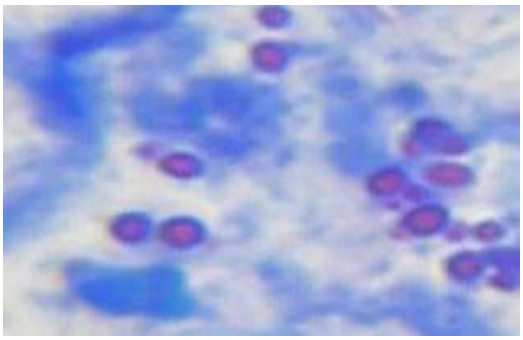

Figure (2): Modified Ziehl-Neelsen in cattle fecal samples (100X). *cryptosporidium* sp.

Table (1): Percentage of infection Samples with *cryptosporidium* spp. in cattle and human

Host	No.samples	No. infection samples	Percentage %	Mean ± SD
Human	150	43	28.66	28.7 ± 3.7
Cattle	150	54	36	36.0 ± 3.9
Total	300	97		

Note: * = Weak significant difference (p is less than 0.05), ** = Moderate (p is less than 0.01), *** = Strong (p < 0.001)

Table (2): Infection rates with *cryptosporidium* spp. from cattle according to sex

sex	No.samples	No.infection samples	Percentage %	Mean ± SD
Female	120	37	30.83	30.8 ± 4.2
Male	30	17	56.66	56.7 ± 9.0
Total	150	54		

* = Weak substantial difference (p < 0.05)

ISSN 2072-3857

Table (3): The percentage of infection with *cryptosporidium* spp. among human samples according to sex

sex	No.samples	No.infection samples	Percentage %	Mean ± SD
Female	58	14	24.13	24.1 ± 5.6
Male	92	30	32.6	32.6 ± 4.9
total	150	34		

* = Weak substantial difference ($p < 0.05$)

Table (4): Percentages of infection with *Cryptosporidium* spp. in human according to regions of basrah province

Region	No. samples	No.infection samples	Samples Infected Percentage %	Mean ± SD
Abu Al Khasib Hospital	25	7	28.0	28.0 ± 9.0
Basra'General Hospital	15	6	40.0	40.0 ± 12.6
Ibn Ghazwan Hospital	50	19	38.0	38.0 ± 6.9
Al-Fayhaa Hospital Teaching hospital	38	9	23.7	23.7 ± 6.9
Almawani hospital	22	2	9.1	9.1 ± 6.1
Total	150	43		

* = Weak substantial difference ($p < 0.05$)

Table (5): The prevalence of *Cryptosporidium* in cattle according to regions

Region	No.cattle samples	No.infection sample	Percentage%	**Mean ± SD**
North of Basrah	37	9	24.32	24.3 ± 7.1
South of Basrah	40	18	45	45.0 ± 7.9
Wast of Basrah	35	16	45.7	45.7 ± 8.4
East of Basrah	38	11	28.94	28.9 ± 7.4
Total	150	54		

* = Weak substantial difference ($p < 0.05$)

ISSN 2072-3857

Table (6): Prevalence of *Cryptosporidium* spp. in human according to age

Age	No.human samples	No. of infection by cryptosporidium	**Mean ± SD**
1-5y	54	20	37.0 ± 6.6
5-18y	46	12	26.1 ± 6.5
18-50y	28	6	21.4 ± 7.8
Over 50	22	5	22.7 ± 8.9
Total	150	43	

* = Weak significant difference ($p < 0.05$)

Table (7): Prevalence of *cryptosporidium* spp. in cattle according to age

Age (month)	No.of samples	No.infection by cryptosporidium	Samples Infected Percentage %	**Mean ± SD**
1-12 m	13	8	61.5***	61.5 ± 13.5
12-36m	73	20	27.4*	27.4 ± 5.2
36-72m	43	18	41.9*	41.9 ± 7.5
72>m	21	4	19.0*	19.0 ± 8.6
Total	150	54		

* = Weak significant difference ($p < 0.05$), *** = Strong significant difference ($p < 0.001$)

Table (8): *Cryptosporidium* spp. oocysts in water in different regions of Basrah province

Region	No.of samples	No.positive sample	**Percentage %**	**Mean ± SD**
South of basrah	13	2	15.4	15.4 ± 10.0
North of basrah	12	1	8.3	8.3 ± 8.0
West of basrah	10	1	10.0	10.0 ± 9.5
East of basrah	15	1	6.7	6.7 ± 6.4
Total	50	4		

* = Weak significant difference ($p < 0.05$)

ISSN 2072-3857

Table (9): *Cryptosporidium* spp. oocysts in soil in different regions of Basrah province:

Region	No.of samples	No.of positive samples	Percentage %	Mean ± SD
South of basrah	10	0	0.0	0.0 ± 0.0
North of basrah	13	1	7.7	7.7 ± 7.4
West of basrah	12	0	0.0	0.0 ± 0.0
East of basrah	15	2	13.3	13.3 ± 8.8
Total	50	3		

* = Weak significant difference ($p < 0.05$)

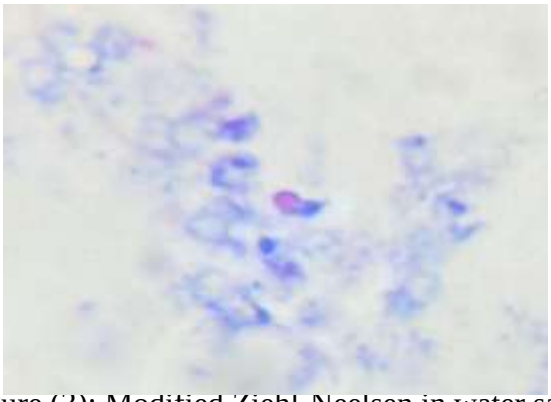

Figure (3): Modified Ziehl-Neelsen in water samples (100X). *cryptosporidium* sp.

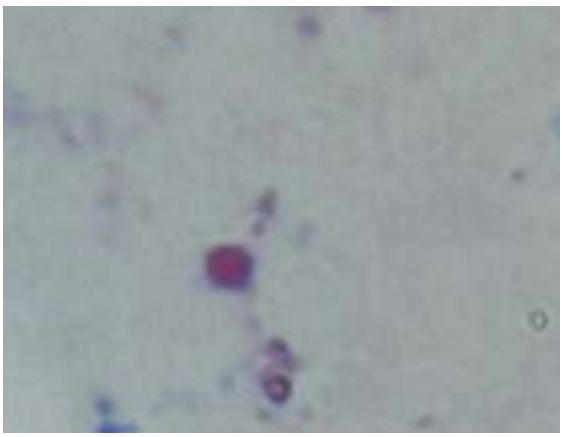

Figure (3): Modified Ziehl-Neelsen in soil samples (100X). *cryptosporidium* sp.

The current study showed a higher prevalence about *Cryptosporidium* infection in cattle compared to humans, which is consistent with several Iraqi studies that have identified cattle as a major reservoir of infection and a significant source of environmental

ISSN 2072-3857

pollution[10, 11]. Comparable findings have been reported worldwide, where livestock were recognized as a key contributor to zoonotic transmission, particularly in rural and agricultural settings[4]. The significant association between infection and sex observed in cattle in the present obtained data is in agreement with previous Iraqi reports suggesting that management practices and exposure levels may influence infection rates between males and females. However, international studies have reported variable results regarding sex-related susceptibility, indicating that such differences may depend on local husbandry conditions and environmental factors. In humans, the absence of a significant difference in infection rates between sexes is consistent with local studies conducted in Basrah and other Iraqi provinces, as well as with several international reports, which suggest that sex alone is not a major determinant of cryptosporidiosis risk. The significant association between infection and sex observed in cattle in the present obtained data is in agreement with previous Iraqi reports, such as those by[18]**,** suggesting that management practices and exposure levels may influence infection rates between males and females. However, international studies, including [19, 20] have reported variable results regarding sex-related susceptibility, indicating that such differences may depend on local husbandry conditions and environmental factors.

In humans, the absence of a significant difference in infection rates between sexes is consistent with local studies conducted in Basrah province **[21]**and other Iraqi regions **[5],** as well as with several international reports such as**[22]**, which suggest that sex alone is not a major determinant of cryptosporidiosis risk.

The significant regional variation observed among human and cattle samples in Basrah province reflects differences in sanitation, water quality, population density, and animal management systems, as previously reported in Iraqi epidemiological investigations [18] [5]. Age was identified as an important factor associated with infection in both humans and cattle, with higher infection rates recorded among young children and young calves. This finding agrees with numerous Iraqi and global studies, including [18], and international works such as **[23]**, which have attributed higher susceptibility in younger age groups to immature immune responses and increased exposure to contaminated environments.

The identification of *Cryptosporidium* oocysts in samples of soil and water, despite the relatively low prevalence, supports The function of environmental contamination during the cycle of transmission of the parasite. Similar observations have been documented in Iraqi environmental studies [5], as well as in international research such as [19, 20], emphasizing importance of monitoring the environment sources to better understand the epidemiology of cryptosporidiosis.

ISSN 2072-3857

References

1. Rojas, C.A., et al., *Species-level characterization of the core microbiome in healthy dogs using full-length 16S rRNA gene sequencing.* Frontiers in Veterinary Science, 2024. **11**: p. 1405470.
2. Stensvold, C.R., et al., *Rodent-adapted Cryptosporidium infection in humans: seven new cases and review of the literature.* One Health, 2024. **18**: p. 100682.
3. Ahmed, A., E.E. Siddig, and N.S. Mohamed, *Arthropod-Borne Zoonotic Parasitic Diseases in Africa: Existing Burden, Diversity, and the Risk of Re-Emergence.* Parasitologia, 2025. **5**(3): p. 29.
4. Weldemariam, D., M. Awoke, and M. Aklilu, *Review on bovine cryptosporidiosis, its associated risk factors and diagnostics methods.* Sm Trop Med J, 2024. **6**: p. 1-08.
5. Al-Saeed, A., J.M. Abdo, and R.G. Al-Simaani, *Cryptosporidiosis in Cattle and Sheep in Duhok City/Kurdistan Region/Iraq.* Kufa Journal for Veterinary Medical Sciences, 2019. **10**(1).
6. Tamrat, H., et al., *Prevalence and associated risk factors of Cryptosporidium infection in calves and hospitalized humans in Libo Kemkem, North Western Ethiopia.* Veterinary Medicine and Science, 2024. **10**(5): p. e70040.
7. Mahmoodi, A., et al., *Study on Soil Contamination with Toxoplasma gondii Oocysts in Eastern Iran.* Archives of Razi Institute, 2025.
8. Lu, H., et al., *Microbial Aerosols in Livestock Farming Environment: A Threat That Cannot Be Ignored.* Veterinary Sciences, 2025. **12**(12): p. 1147.
9. Zubair, A.I. and A.A. Faraj, *Molecular identification of Giradia intestinalis in human and cats in Dohuk city, Kurdistan Region-Iraq.* Diyala Journal for Veterinary Sciences, 2025. **3**(1): p. 105-119.
10. Saleh, F.E.-z.R., H.H. Abdullah, and D. Aboelsoued, *Coprological and molecular prevalence of Cryptosporidium and Giardia in cattle and irrigation water from Beni-Suef Governorate, Egypt.* Scientific Reports, 2025. **15**(1): p. 26983.
11. Abdulqader, S., et al., *Cryptosporidium parvum screening in young calves with diarrhoea in Sulaymaniyah Governorate, Iraq.* Veterinární medicína, 2025. **70**(2): p. 45.
12. Felefel, W., et al., *Molecular identification and histopathological of Cysticercus tenuicollis infestation among small ruminants.* Iraqi Journal of Veterinary Sciences, 2025. **39**(2): p. 377-389.
13. Hassan, N.J., *Climate Change and Trend Analysis of Temperature: The Case of Sulaymaniah Region, Iraq.* Journal of Babylon Center for Humanities Studies, 2025. **15**(1).
14. Fenta, M.D., et al., *Foodborne and neglected parasitic zoonoses in Ethiopian red meat animals: insights from a systematic review and meta-analysis.* Frontiers in Veterinary Science, 2025. **12**: p. 1501940.
15. Zajac, A.M., et al., *Veterinary clinical parasitology*. 2021: John Wiley & Sons.
16. Ljungström, S., et al., *Comparison of four diagnostic methods for detection and relative quantification of Haemonchus contortus eggs in feces samples.* Frontiers in veterinary science, 2018. **4**: p. 239.
17. Lamba, J., K. Karthikeyan, and A. Thompson, *Apportionment of suspended sediment sources in an*

agricultural watershed using sediment fingerprinting. Geoderma, 2015. **239**: p. 25-33.
18. Mahdi, N. and N. Ali, *Intestinal parasites, including Cryptosporidium species, in Iraqi patients with sickle-cell anaemia.* EMHJ-Eastern Mediterranean Health Journal, 8 (2-3), 345-349, 2002, 2002.
19. Fayer, R., *Cryptosporidium: a water-borne zoonotic parasite.* Veterinary parasitology, 2004. **126**(1-2): p. 37-56.
20. Smith, H.V. and R.A. Nichols, *Cryptosporidium: detection in water and food.* Experimental parasitology, 2010. **124**(1): p. 61-79.
21. Mahdi, N. and N. Ali, *Cryptosporidiosis among animal handlers and their livestock in Basrah, Iraq.* East African medical journal, 2002. **79**(10): p. 551-554.
22. Hunter, P.R. and G. Nichols, *Epidemiology and clinical features of Cryptosporidium infection in immunocompromised patients.* Clinical microbiology reviews, 2002. **15**(1): p. 145-154.
23. Budge, S., et al., *Environmental enteric dysfunction and child stunting.* Nutrition reviews, 2019. **77**(4): p. 240-253.

ISSN 2072-3857