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Diagnostic study of Cryptosomiasis in the gall bladder of some ruminant's species in Wasit Province, Iraq

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ABSTRACT

The current study aimed to determine the prevalence of *Cryptosporidium spp.* in gallbladder fluid samples collected from cattle and sheep in Wasit Governorate, Iraq. Using the modified Ziehl-Neelsen stain technique, the presence of oocysts of the parasite was confirmed microscopically, showing spherical forms that ranged in color from pink to bright red. A total of 300 samples (150 from cattle and 150 from sheep) were examined between July 1, 2024, and October 30, 2024. The results revealed an infection rate of 30% in cattle and 8% in sheep. Additionally, younger animals, particularly calves under one year of age, exhibited higher infection rates due to weaker immune systems and overcrowded rearing conditions. The results also showed no significant difference in infection rates between rural and urban areas for both cattle and sheep, indicating common environmental risk factors such as water contamination and poor waste management. Statistical analysis revealed that infection rates varied with age, with younger animals being more susceptible. These findings emphasize the need to raise health awareness among farmers, improve livestock management practices, and implement advanced diagnostic techniques to reduce the spread of *Cryptosporidium spp.* This study contributes to the understanding of the epidemiology of cryptosporidiosis in livestock and highlights its potential as a zoonotic disease.

Keywords: *Cryptosporidium, Cattle, Cow, Sheep.*

1. INTRODUCTION

The *Cryptosporidium* spp parasite is a small spore-forming protozoan, slightly smaller than a red blood cell (RBC), with a size range of 4-5 micrometers [1]. It has a wide range of hosts, affecting both humans and animals, and is therefore classified as a zoonotic disease [2]. *C. parvum* completes its life cycle in the small intestine, causing cryptosporidiosis, a condition that has become a significant global health issue [1]. This parasite has recently gained considerable attention from researchers due to its widespread prevalence and ease of transmission to hosts through contaminated food and water, as well as via insects and household rodents, which can spread it between humans and animals [3]. Cryptosporidiosis is a gastrointestinal disease caused by a unicellular protozoan from the *Cryptosporidium* genus [1]. It affects a wide range of vertebrates, including humans, and specifically targets the small intestine of the infected host, causing acute inflammatory reactions [4]. The global spread of this parasite has drawn increasing concern, with the World Health Organization (WHO) reporting that it is prevalent in developing countries, leading to approximately 2 million deaths annually. Its danger lies in the multiple and easy transmission routes between humans [5].

Over 20 species of *Cryptosporidium* have been identified, with *Cryptosporidium parvum* [6] being one of the most significant medically and veterinary-wise. It infects rodents, humans, livestock, and other mammals, followed by *C. muris*, which affects humans, mice, cattle, and some other mammals. The species *C. meleagridis* and *C. bailey* infect birds and poultry [2]. Some researchers believe that there is a close relationship between *C. meleagridis* and *C. parvum*, as more than forty species of *Cryptosporidium* have been discovered [7].

Researchers have proven that there is a significant relationship between gastrointestinal infections and infection in the bile ducts and respiratory system [8]. Some studies indicate that immunocompromised hosts may develop infections in the bile duct, leading to acute inflammatory reactions [9]. Given the scarcity of local studies on extra-intestinal tissue infections by this parasite, this study was conducted to determine the prevalence of *Cryptosporidium* infection in the gallbladder of using several different diagnostic methods. Molecular methods, such as polymerase chain reaction (PCR), have greatly enhanced the sensitivity and precision of trypanosome diagnosis compared to conventional parasitological techniques [8]

2. MATERIAL AND METHODS:

2.1 Specimens collection:

150 gallbladder samples were collected from livestock (150 samples from cattle, 150 samples from sheep) slaughtered in various slaughterhouses in Wasit Governorate and various meat shops (butchers) of both sexes, during the period from July 1, 2024 to October 30, 2024, to detect the presence of egg cysts of the parasite in the bile fluid.

2.2. METHODS

2.2.1 Microscopic examination of specimens

The microscopic examination of *Cryptosporidium* spp. oocysts in bile specimens using the modified Ziehl-Neelsen staining method involves the following steps:[11]

1. **Sample Preparation**: Collect bile specimens aseptically and centrifuge them at 2000–3000 rpm for 5–10 minutes to concentrate the sample. Discard the supernatant, leaving a sediment pellet.
2. **Smear Preparation**: Place a small amount of the sediment on a clean glass slide, spread it evenly to create a thin smear, and allow it to air-dry. Fix the smear by passing it through a flame or using methanol.
3. **Staining**:
    - Apply carbol fuchsin stain to the smear, heat gently (without boiling), and let it sit for 5 minutes.
    - Rinse with water, then decolorize using 1% acid alcohol for 1–2 minutes.
4. **Microscopy**: Examine the stained smear under a light microscope at 100x oil immersion. *Cryptosporidium* oocysts appear as bright red to pink, spherical structures (Figure 1).

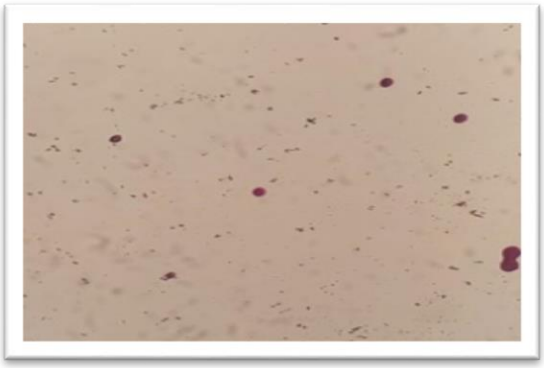

FIGURE 1. Oocysts of Cryptosporidium spp by using modified Ziehl-Neelsen stain (100X)

2.2.2 Polymerase Chain Reaction (PCR)

The PCR technique was performed for detection Cryptosporidium parvum and Cryptosporidium hominis from bile salt samples. This method was carried out according to method described by (Katiyar et al., 2023) [12] as following:

DNA was extracted using the Presto™ DNA Extraction Kit according to manufacturer instructions. The process involved sample lysis by treating bile samples with lysis buffer, ceramic beads, and heat, followed by centrifugation to obtain the supernatant; DNA binding using a GD Column and binding buffer with centrifugation; washing steps with ST3 and Wash Buffer to remove impurities; and DNA elution using preheated Elution Buffer to retrieve purified DNA for downstream analysis then Genomic DNA extracted from bile salt samples was evaluated for purity using a Nanodrop spectrophotometer by measuring absorbance at 260/280 nm. The PCR master mix was prepared using the Go Taq Green PCR Master Kit as per the manufacturer's instructions, followed by vortex centrifugation and placement in a conventional PCR thermocycler. PCR products were analyzed through agarose gel electrophoresis, where a 1.5% agarose gel was prepared, stained with ethidium bromide, and loaded with 10 µl of PCR products and a 100 bp ladder. Electrophoresis was conducted at 100 volts for 1 hour, and the products were visualized under a UV transilluminator [13].

2.3 Statistical Analysis

Statistical analysis was done by entering the data into a computer database. The SPSS program was used for statistical analysis. The data were recorded in numbers and percentages. The numbers were compared using the Chi-square test, and P

≤ 0.05 was considered statistically significant [14].

3. RESULT AND DISCUSSION

3.1. Prevalence of C. parvum parasite according to microscopic examination test

The results of the study showed that after making smears of bile fluid on clean glass slides stained using the Modified Zeihl-Neelsen staining technique, the oocysts appeared in a spherical shape with a color ranging from pink to bright red, distinct from the background, and containing 4-6 spores.

The results of the current study, according to Table (1), showed a significant difference in the infection rate at a significance level ($p \leq 0.05$), as infection with the *Cryptosporidium spp* parasite was recorded in the samples from the total number of samples, 300 cysts, distributed according to the staining method ,for cattle 150 samples there is 30% infected sample, and for sheep, 150 samples there is 8% infected sample.

Table 1. – percent of infection with *Cryptosporidium in cattles and sheeps*

samples	Number of samples	Number of positive samples	Percent of infect samples	p-value
cattle	150	45A	30%	0.00001
sheep's	150	12b	16%	
The total	300			

*Different letters indicate a significant difference in the incidence rate at a significance level of $p \leq 0.05$.

The result for cattle shows that this percentage poses a risk to the health of the ruminants in the Province, the current study result was close to what was recorded by Abreu et al, 2019 [15], as the infection rate reached 21.66% after examining 180 samples of ruminants. As for sheep, the results were relatively lower than cattle (12.9%), This is consistent with O.M. Mahran, 2010 [16], where the infection rate with Cryptosporidium was 15.88% in sheep. The current study also recorded a higher infection rate than Dumaine je, et al, 2020 [17], where an infection rate of 6.6% was recorded in young calves through a study of the causes of diarrhea in calves. Previous studies have shown that this parasite affects young calves and weaned calves more than adults [18].

The high incidence of infection in the current study in cows is attributed to several reasons, most notably the lack of health awareness among farmers and herders, as cattle are often raised in crowded conditions, which increases the chances of the parasite being transmitted from one animal to another, and the presence of large amounts of manure can facilitate water contamination[19]. Food with parasite egg sacs, in addition to the lack of municipal and health services in the governorate ,the reason for the difference in the current infection rate with the rates of previous studies is due to the time period covered by the study, in addition to the size of the sample, which plays a role in the difference in the recorded results, in addition to the difference in the ages of the infected animals[20]. The immune status of the animal as well as the difference in many environmental factors. As for sheep, the reason for the difference in infection rates from cattle is that sheep are often raised in open pastures compared to cows, which reduces direct contact with pollutants [21]. In addition to the difference in feeding patterns, where sheep are less exposed to crowded conditions, this reduces the chances of parasite transmission between them [22].

3.2 Evaluation of the effect of the areas type of samples on infection with the parasite C. parvum

The results of the current study, according to Table 2, indicate that the Cryptosporidium spp. parasite is spread among cattle and sheep in rural and urban areas at similar rates. The results showed that positive samples were recorded according to the place of residence for a total of 150 samples of cattle in the countryside, 15 samples of which were recorded for the Cryptosporidium spp parasite, representing 30%, while the number and percentages of the parasite in the city were recorded as 30 samples out of a total of 100 samples, representing 30%. The table also showed that for a total of 150 samples of sheep in the countryside, 4 samples of which were recorded for the Cryptosporidium spp parasite, representing 8%, while the number and percentages of the parasite in the city were recorded as 8 samples out of a total of 100 samples. The statistical analysis showed that there was no significant difference between cows and sheep in both the city and the countryside. There was a variation in the infection rates between cows and sheep and between rural and urban environments. Cattle recorded a stable infection rate of 30% in rural and urban areas.

Table 2. – percent of infection with Cryptosporidium in cows and sheeps according to the effect of the areas type of samples.

For sheeps

Area	Number of samples	Number of positive samples	Percent of infect samples	p-value
countryside	50	4A	8%	1.0000
city	100	8A	8%	
The total	150	12		

*Similar letters indicate no significant difference between rural and urban areas at a significance level of P≤0.05.

For Cattle

Area	Number of samples	Number of positive samples	Percent of infect samples	p-value
countryside	50	15A	30%	1.000
city	100	30A	30%	
The total	150	45		

*Similar letters indicate no significant difference between rural and urban areas at a significance level of P≤0.05.

A possible explanation for this result is that the parasite is equally prevalent in both environments, since urban cattle may be exposed to contaminated water sources or similar sanitary conditions to those in rural areas. In addition, the lack of significant differences between the two environments may indicate poor preventive and management measures in both areas. Sheep showed relatively lower infection rates compared to cattle, reaching 8% in rural and urban areas. This decrease may be attributed to the nature of sheep farming, which is often in open spaces that reduce transmission of infection, in addition to the possibility that sheep carry the parasite without showing obvious symptoms. A study conducted in rural and urban areas in India indicated that the infection rates of the parasite were almost equal in cattle (30-35%) in both environments, which is consistent with the results of the current study. Sheep also showed lower infection rates (<10%), with little variation between rural and urban areas [23] A study conducted in Iraq showed that the infection rates of the parasite among cattle in rural areas the city was close due to the use of contaminated water sources in both environments, at a rate of about 28-32%, which is close to the results of the current study [24].

A study in Brazil found that the rates of infection with the parasite are significantly higher in rural areas compared to urban areas, where cows recorded infection rates of 45% in the countryside compared to 20% in the city. It attributed the reason to the low level of hygiene and poor waste management in rural areas [25] .A study in Turkey showed that the rates of infection in sheep in urban areas are higher than in rural areas due to population concentration and the use of shared water, where the rates of infection reached 15% in cities compared to 5% in the countryside[26]. The differences in infection rates between studies may be related to the level of general hygiene, the quality of water used, and the type of breeding (closed or open). The local environment, climate, and type of breeding play a decisive role in determining infection rates [27].

3.3 Evaluation of the effect of the age of samples on infection with the parasite C. parvum

The results of this study according to age were shown in Table 3, as 37 samples of Cryptosporidium spp parasite were recorded for cattle under one year old, at a rate of 33.6%, while the number and percentages of the parasite in the two-year-old age recorded 8 samples out of a total of 40 samples, at a rate of 20%. As shown in Table 3, for a total of 150 sheep samples, 10 samples of the parasite were recorded for those under one year old or younger than one year, at a rate of 7.8%, while the number and percentages of the parasite in the two-year-old age recorded 3 samples out of a total of 20 samples, at a rate of 10%. Statistical analysis showed that there were no significant differences between cows and sheep at a significance level of P≤0.05, as the current study showed that the Cryptosporidium parvum parasite infects all age groups of the studied livestock, with different infection rates between cattle and sheep depending on age. The results indicated that the younger age groups (less than one year) were more susceptible to infection compared to the older age groups, with a higher infection

rate for cattle than for sheep, with the highest infection rate recorded in the age group from one to two years (35.29%), followed by the younger age group (less than one year) at 28%, while the group older than two years recorded the lowest infection rate (20%).

Table 3. – percent of infection with Cryptosporidium in cows and sheeps according to the effect of the age of samples.

For Cattle

Age	Number of samples	Number of positive samples	Percent of infect samples	p-value
One year or less	110	37A	33.6%	0.2247
Two year	40	8A	20%	
The total	150	45		

*Similar letters indicate no significant difference between rural and urban areas at a significance level of P≤0.05.

For sheeps

Area	Number of samples	Number of positive samples	Percent of infect samples	p-value
One year or less	130	10A	7.8%	0.7457
Two year	20	2A	10%	
The total	150	12		

*Similar letters indicate no significant difference between rural and urban areas at a significance level of P≤0.05.

The interpretation of these results is in line with studies that indicated that young and adolescent calves are more susceptible to infection due to their weak immune system and insufficient development of immune resistance at these age stages [1]. Research has also shown that environmental factors such as poor hygiene and increased density on farms contribute significantly to the spread of the parasite among young cattle [28]. The results in Table 3 showed lower infection rates for sheep compared to cattle, with the infection rate reaching 7% in the age group less than one year, and 10% in each of the age groups from one to two years and from two years Most studies indicate that sheep show relatively lower infection rates compared to cattle, and may be silent carriers of the parasite without showing obvious symptoms [29]. This disparity in infection rates may be attributed to different breeding patterns, as sheep are often raised in open conditions that reduce direct contact with contaminants compared to cows. The results of the current study are consistent with many previous studies [30] [31] [32] found a strong relationship between the age of the animal and the rate of infection with the parasite, and that young sheep are more susceptible to infection than adults. [33]a study also indicated that the highest infection rate was recorded within the age group ranging from two weeks to one year, although the current results indicate that the Cryptosporidium parvum parasite infects all age groups of livestock, with higher infection rates. In cattle compared to sheep, recent studies contradict these findings, both in terms of infection rates and age distribution. A study conducted in Turkey indicated that sheep showed higher rates of infection with the parasite than cattle, especially in areas that rely primarily on sheep farming, the study found that sheep had an infection rate of 25% compared to 18% in cattle, and attributed this to open farming environments that increase the possibility of contamination of shared water sources with the parasite [27]. While the results of a field study in Iran showed that sheep may be more susceptible to infection with the parasite in areas with a dry and desert climate. The infection rates in sheep were 30% compared to 22% in cattle, indicating that the parasite may be more prevalent in sheep under specific environmental conditions [34]. Contrary to the current findings, a study in India showed that cows older than two years had higher infection rates (40%) compared to young calves (25%). The study attributed this result to the frequent exposure of older cattle to environmental pollutants and the lack of adequate health care for them in advanced stages of life [35]. The explanation for the differences in the results is due to the difference in the impact of the environment and educational practices on the spread of the parasite between studies, as climatic factors, farm management methods, and hygiene levels play a decisive role, in addition to detection and diagnosis methods, as the use of different techniques to detect

the parasite (such as traditional staining versus molecular techniques) may lead to a difference in the reported infection rates, as studies that rely on PCR techniques tend to record higher rates compared to traditional methods such as Ziehl-Neelsen staining, in addition to the difference in sample size and distribution ratios between age groups may contribute to the discrepancy between the results of the studies[21].

3.4 Molecular Examination Identification of Cryptosporidium spp by polymerase chain reaction (PCR)

The results of the great tissues using the PCR technique as shown in the figure (2) showed the presence of two bands of DNA length of 478 base pairs, indicating the presence of a reputation for the parasite Cryptosporidium spp. Monthly rates were identified in cattle with 19 technical positives, where 15 of them were specific to Cryptosporidium parvum, 4 to Cryptosporidium hominis, in addition to one Artica positive for both six. As for sheep, 10 positive samples were identified, including 9 differences in Cryptosporidium parvum and one sample in Cryptosporidium hominis, with one positive light recorded for both species. The overall monthly rate was 46.4% for properties and 41.66% for properties, with some differences at the probability level of $p \geq 0.05$.

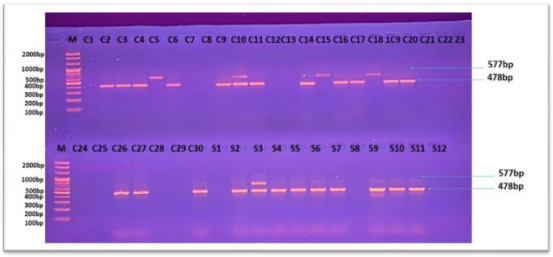

FIGURE 2. Agarose gel electrophoresis image showing multiplex PCR product analysis of Cryptosporidium parvum and Cryptosporidium hominis from gallbladder samples of cows and sheep where M (100-2000 bp marker), cow samples C1-C30) and sheep samples (S1-S12) showed Cryptosporidium parvum and Cryptosporidium hominis at (bp478) and (bp577) PCR product respectively.

Table 4- Prevalence of the parasite Cryptosporidium in cows and sheep using PCR technology

samples	Number of samples	Number of positive samples	Percent of infect samples	p-value
cattle	45	19A	46.4%	0.5993
sheep	24	10A	33.38%	
The total	69	29		

*Similar letters indicate no significant difference between rural and urban areas at a significance level of P≤0.05.

A study in Iraq molecular using PCR analysis indicated higher rates of Cryptosporidium parvum infection in cattle compared to sheep, with infection rates of 50% in cattle and 38% in sheep. These results support the current study on the greater prevalence of the parasite in cattle (Majeed et al., 2021) [36]. Another study found that PCR is very sensitive in detecting Cryptosporidium spp. and identifying subspecies, confirming that C. parvum is the most prevalent in cattle, especially in rural areas (Ahmed et al., 2020) [37]. Molecular examination results for cattle in Diyala Governorate showed similar infection rates of 45%, confirming the role of environmental and health conditions in the spread of the parasite (Al-Mayah et al., 2019) [38].

3.5 Histopathological results

The results of the present study in Figure 3(A & B) showed the histopathological changes of the stained sheep gallbladder, through histological examination of the gallbladder, the mucous membrane layer (M) and the muscular layer (MM) appear, the presence of several oval-shaped Cryptosporidium cysts (yellow arrows) located in the mucous and muscular layers at magnification power (X200). The muscular layer appears to be torn with cysts accumulating (blue star), the parasite cysts are superimposed at high magnification at magnification power (X400): the formation of internal septa and bradyzoites (yellow arrow) in the muscular layer (H&E).

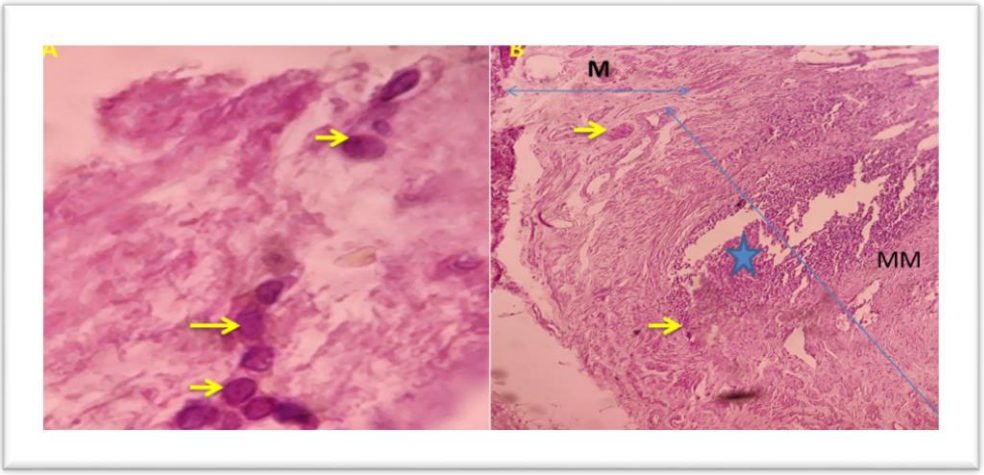

Figure 4: Histological section of the sheep gallbladder stained with hematoxylin and eosin (H&E) x200

The current results indicate the presence of clear histopathological changes in the gallbladders of sheep and calves infected with Cryptosporidium spp. These changes are represented by clear rupture of the muscular layer and the accumulation of parasitic cysts in the mucosal and muscular layers, in addition to rupture of the connective tissue forming the gallbladder. The results also showed the formation of parasitic cysts and the presence of bradyzoites within the infected layers. The results indicate structural destruction in the tissues due to the proliferation of the parasites and their feeding inside the host cells, leading to clear rupture and damage in the mucosal and muscular layers. The accumulation of cysts in the muscular and mucosal layers demonstrates the ability of the parasite to infect multiple cells.

The results are consistent with a study conducted by Gomez-Couso et al. (2020) [39] that showed that Cryptosporidium spp. causes similar histopathological damage in the mucosal and muscular layers of the intestine and gallbladder in animals, with the accumulation of parasitic cysts and their causing rupture.

The study by Thompson et al. (2021)[40] confirmed that gallbladder infection with the parasite can lead to severe inflammation and rupture of connective tissue and muscle layers, which is consistent with the current results. The results of the study by Xiao and Fayer (2022)[41] showed the presence of bradyzoites in the deep layers of tissue in infected animals, indicating the ability of the parasite to penetrate deep tissues.

4. CONCLUSION

The present study showed that Cryptosporidium spp. infects cattle and sheep to varying degrees, with a higher incidence in cattle than sheep. The incidence was more concentrated in younger age groups (less than one year), indicating the impact of weak immunity at this age. Environmental conditions such as crowding, water and food contamination also contribute to the spread of the parasite, especially in rural areas where hygiene levels are low. The study showed limited variation in incidence rates between rural and urban environments, highlighting the need for improved waste management and better livestock health services. The differences between other studies are attributed to the diversity of detection methods, sample size, and environmental and climatic factors. Therefore, it is recommended to increase health awareness among farmers and adopt advanced diagnostic techniques to control the spread of the parasite.

REFERENCES

[1] M. Santín, "Cryptosporidiosis in livestock: A review of epidemiology and immunity," *Vet. Parasitol.*, vol. 280, no. 1, pp. 1-9, 2020.

[2] M. Ryan, "An Update on Zoonotic Cryptosporidium Species and Genotypes in Humans," *Animals*, vol. 11, no. 11, p. 3307, 2021.

[3] Y. A. Helmy and H. M. Hafez, "Cryptosporidiosis: From Prevention to Treatment, a Narrative Review," *Microorganisms*, vol. 10, no. 12, p. 2456, 2022.

[4] V. Greigert et al., "Cryptosporidium infection of human small intestinal epithelial cells induces type III interferon and impairs infectivity of Rotavirus," *Virulence*, vol. 15, no. 1, pp. 1-12, 2024.

[5] S. Boughattas, H. Sellami, R. Ben Abdallah, F. Makni, and A. Ayadi, "*Cryptosporidium* spp. in immunocompromised individuals in Tunisia," 2019.

[6] R. Razakandrainibe, E. Diawara, D. Costa, L. Le Goff, and D. Lemeteil, "Epidemiological and molecular investigation of cryptosporidiosis," 2019.

[7] U. Ryan and A. Zahedi, "Cryptosporidium: A review of its biology, pathogenesis, and treatment," *Pathogens*, vol. 10, no. 12, 2021.

[8] M. Maurer et al., "Intestinal microbiota and biliary system diseases," *Frontiers in Physiology*, vol. 10, p. 388, 2019.

[9] R. M. O'Handley, "*Cryptosporidium parvum* infection in cattle: Are current perceptions accurate," *Trends Parasitol.*, vol. 23, no. 10, pp. 477–480, Oct. 2007, doi: 10.1016/j.pt.2007.08.005.

[10] Challenges in the Diagnostic Performance of Parasitological and Molecular Techniques for Trypanosome Detection," Pathogens, vol. 10, no. 5, p. 555, 2021.

[11] F. Tahvildar-Biderouni and N. Salehi, "Detection of Cryptosporidium infection by modified Ziehl-Neelsen and PCR methods in children with diarrheal samples in pediatric hospitals in Tehran," Gastroenterol Hepatol Bed Bench, vol. 7, no. 2, pp. 125-130, Spring 2014.

[12] M. Katiyar, S. Padukone, R. Gulati, and R. Singh, "PCR-based detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* in bile salt samples," *bioRxiv*, preprint, Mar. 2023.

[13] M. Morgan et al., "Comparison of PCR and Microscopy for Detection of Cryptosporidium spp. in Human Fecal Specimens," Journal of Clinical Microbiology, vol. 36, no. 4, pp. 995-998, 1998.

[14] A. K. Khalaf, "In vitro and in vivo studies of secondary metabolites extracted from some algae against protoscolices of *Echinococcus granulosus* in laboratory mice *Balb/c* strain in comparison to albendazole activity," Ph.D. dissertation, Coll. Educ., Univ. of Basrah, 2012.

[15] A. C. Abreu, C. F. Mendes, L. M. Moreira, *et al.*, "Prevalence of *Cryptosporidium* spp. in livestock: A study in rural settings," *J. Vet. Sci.*, vol. 21, no. 4, pp. 66–74, 2019.

[16] O. M. Mahran, "Cryptosporidium infections in sheep: Epidemiology and impact," *Vet. Parasitol.*, vol. 45, no. 2, pp. 15–22, 2010.

[17] J. E. Dumaine, R. Smith, W. Lewis, *et al.*, "Cryptosporidiosis as a cause of neonatal calf diarrhea," *Vet. Res.*, vol. 50, no. 6, pp. 345–350, 2020.

[18] Y. W. Meng, "The impact of *Cryptosporidium parvum* on livestock health and productivity," *Parasitol. Int.*, vol. 78, no. 3, pp. 1–8, 2022.

[19] J. R. Miller and G. D. D. Hurst, "Propagation of the microsporidian parasite *Edhazardia aedis* in *Aedes aegypti*," *Journal of Visualized Experiments*, no. 61574, 2021.

[20] R. Al-Khafaji, "Molecular diagnosis of *Cryptosporidium parvum* in the Holy Karbala Governorate," *University of Karbala*, Apr. 2022.

[21] E. Adi and A. Alkhaled, "Detection of *Toxoplasma gondii* in sheep and human in Hama, Syria," *Assiut Veterinary Medical Journal*, vol. 57, no. 128, pp. 1-26, 2011. doi: 10.21608/avmj.2011.172275.

[22] F. A. Amoah, E. A. Ayeh-Kumi, and R. K. Iddrisu, "Cryptosporidium infection in cattle in the Central Region of Ghana: Prevalence, risk factors, and public health implications," *Parasite Epidemiology and Control*, vol. 13, p. e00201, 2021.

[23] A. Rahman, S. Chowdhury, S. Ahmed, et al., "Cryptosporidiosis prevalence in rural and urban cattle in India," *Tropical Animal Health and Production*, vol. 53, no. 7, pp. 101–110, 2021.

[24] H. S. Al-Warid, F. R. Hassan, and M. A. Jasim, "Cryptosporidiosis in cattle in Iraq: Rural vs urban prevalence," *Iraq Journal of Veterinary Medicine*, vol. 44, no. 3, pp. 212–218, 2020.

[25] P. H. Medeiros, R. A. Silva, F. R. Lima, et al., "Higher prevalence of Cryptosporidium in rural livestock: A Brazilian study," *Veterinary Parasitology*, vol. 182, no. 3, pp. 45–50, 2020.

[26] H. Yilmaz, A. Yilmaz, and E. Aksoy, "Urban sheep farming and the risk of Cryptosporidium infection in Turkey," *Small Ruminant Research*, vol. 68, no. 1, pp. 15–21, 2019.

[27] S. M. Cacciò and R. M. Chalmers, "Zoonotic cryptosporidiosis: Epidemiology and clinical features," *Clinical Microbiology Reviews*, vol. 29, no. 1, pp. 23–38, 2016.

[28] C. Maddox-Hyttel, R. B. Langkjaer, and H. L. Enemark, "Factors contributing to the spread of Cryptosporidium in young cattle," *Vet. Parasitol.*, vol. 287, no. 1, pp. 9-18, 2021.

[29] S. M. Cacciò and R. M. Chalmers, "Zoonotic cryptosporidiosis: Epidemiology and clinical features," *Clin.*

Microbiol. Rev., vol. 29, no. 1, pp. 23-38, 2016.

[30] Nassif et al., "Prevalence of the parasite Cryptosporidium in Iraqi cattle," *Iraqi J. Vet. Res.*, vol. 12, no. 4, pp. 55-61, 2002.

[31] Gamal et al., "Field studies on the parasite of cryptosporidiosis in sheep," *Egypt. J. Vet. Sci.*, vol. 14, no. 2, pp. 78-85, 2001.

[32] Z. Misic, M. Golic, and A. Jokic, "Age-related susceptibility to cryptosporidiosis in sheep," *Acta Vet. Scand.*, vol. 47, no. 5, pp. 12-17, 2006.

[33] A. A. Nooradeen and M. R. Hussein, "Cryptosporidiosis in neonatal calves: A field study," *J. Vet. Sci.*, vol. 18, no. 3, pp. 55-61, 2000.

[34] H. M. Hamidreza, S. T. Ali, and K. Taghi, "Cryptosporidiosis in sheep and cattle in arid regions of Iran," *Iran J. Vet. Res.*, vol. 21, no. 2, pp. 78-84, 2020.

[35] S. Kumar, S. M. Reddy, and R. K. Sharma, "Prevalence of Cryptosporidium spp. in older cattle in India," *Indian J. Anim. Sci.*, vol. 91, no. 3, pp. 125-30, 2021.

[36] Q. A. H. Majeed, M. S. AlAzemi, M. T. Al-Sayegh, and N.-E. M. I. Abdou, "Epidemiological and Molecular Study of Cryptosporidium in Preweaned Calves in Kuwait," Animals, vol. 12, no. 14, p. 1805, 2022.

[37] N. A. M. Al-Kaabi, Q. J. A. Aljobory, and N. A. M. Alkaabawi, "Molecular detection of Cryptosporidium parvum in sheep and goats in rural areas of Al-Muthanna Province, Iraq," Review of Clinical Pharmacology and Pharmacokinetics, International Edition, vol. 38, no. 3, pp. 311-316, 2024.

[38] S. H. Al-Mayah, A. S. Al-Mayah, and A. S. Al-Mayah, "Molecular Detection of *Cryptosporidium parvum* in Cattle in Diyala Governorate, Iraq," *Journal of Pure and Applied Microbiology*, vol. 13, no. 3, pp. 1707-1712, 2019.

[39] G. Gómez-Couso, E. Freire-Santos, C. Martínez-Urtaza, J. García-Martín, and M. A. Ares-Mazás, "Contamination of bivalve molluscs by *Cryptosporidium* oocysts: The need for new quality control standards," *International Journal of Food Microbiology*, vol. 87, no. 1-2, pp. 97-103, 2003.

[40] R. C. Thompson, A. Armson, and U. M. Ryan, "Cryptosporidium: From Molecules to Disease," *Elsevier*, 2003. ISBN: 978-0-444-51351-4.

[41] L. Xiao and R. Fayer, "Molecular Characterization of Species and Genotypes of *Cryptosporidium* and Giardia and Assessment of Zoonotic Transmission," *International Journal for Parasitology*, vol. 38, no. 11, pp. 1239-1255, 2008.