Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN:2410-8863
E-ISSN:2958-6178
Vol. 1, No. 4, December 2023

Clinical, Infection Rate and Conventional Identification of Cryptosporidium spp. in Children , Lambs and Goat Kids

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Abstract:

Background:

Cryptosporidiosis is considered to be a dangerous zoonotic infection brought on by a protozoan parasite with detrimental economic and public health repercussions on both humans and animals.

Aims: The study aimed to clinical, infection rate and identification with morphological characterization of Cryptosporidium spp from Children, lambs and kids.

Material and Methods: A total of 200 fecal samples was collected from 100 children, 65 lambs and 35 goat kids from different regions in Diyala governorate, were collected from September 2022 to May 2023 and traditional techniques (Sheather's flotation technique, modified ziehl-neelsen staining microscopic examination) were used to check for Cryptosporidium spp. infection.

Results: Clinical symptoms of cryptosporidium infection in 53 lambs and 15 goat kids included watery diarrhea, anorexia, sadness, abdominal discomfort, and weight loss. As a result, it was determined that the total infection rate was 45.5% (89/200), which was made up of 81.5% (53/65) in lambs, 42.9% (15/35) in goat kids, and 21% (21/100) in children. There was a significant difference ($P < 0.05$) in the infection rate of cryptosporidiosis between sexes, as shown by high infection in males (72.7%), females (65%) and goat kids, respectively, in the age groups of less than 3 months (66.7%), more than 3 months (51.4%), and less than 3 months (40.0%). Regarding the area, the majority of infected children lived in the rural area at a ratio of 27.7%, followed by the urban area at a ratio of 13.04%. Further analysis of the relationship between study months and infection rates revealed that the highest infection rates 33.3%, 62.5%, and 66.7% were seen in November, while the lowest infection rates 5%, 50%, and 33.3% were observed in May, in Children, lambs, and kids, respectively.

Conclusion, this study showed the significance of Cryptosporidium in lambs as opposed to youngsters and children, where a high infection rate and the relationship between sex, age groups, system houses, and months of study had a major impact on the infection rate of Cryptosporidiosis.

Key words: Clinical, Infection rate ,Cryptosporidiosis, Lambs, Goat Kids, Children.

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Introduction

Cryptosporidiosis is a newly discovered zoonotic illness and intracellular protozoal parasite that affects both people and animals and causes extraintestinal and gastrointestinal problems. Although the condition is well-known in veterinary medicine, it has just lately come to light as a major protozoal source of diarrhea in people [1]. Animal handlers, children in daycare, immunosuppressive treatment patients, and others with chronic illnesses are all at risk [2,3]. Cryptosporidium is a genus of protozoan parasites that infects a wide range of vertebrates, including people and animals, and is thought to be the main cause of diarrhea in children [4]. It causes substantial morbidity and death in both the developing and industrialized worlds. According to Robertson *et al.* 2010 [5] the majority of zoonotic Cryptosporidium that people get from tainted food and water or close contact with animals comes from ruminants. Faecal-oral contact or consumption of food or water tainted with Cryptosporidium oocysts are the two main ways that Cryptosporidiosis is spread [6]. According to reports, pediatricians in rural and semi-urban regions have a greater risk of Cryptosporidium species infection than pediatricians in urban areas [7,8,9]. Others said there was no discernible difference in prevalence rates in urban and rural areas [10]. The prevalence of children 1 year of age or below with Cryptosporidium-associated diarrhea is higher in rural and suburban areas, and it negatively correlates with age [10,11,12].

Although most Arab countries are characterized by Infection with Cryptosporidium seems to be more common during the rainy months that are often connected to the cold season of the year [9,12], despite the fact that we have a hot summer and a moderate winter. Diarrhea is brought on by the protozoan Cryptosporidium, which affects both people and animals. They often cause diarrhea in both immunocompetent and immunocompromised individuals

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worldwide, which has a serious detrimental impact on morbidity and mortality, especially in impoverished countries and in young children under the age of five [13].

In Iraq, Rasheed, 1997 [14] The prevalence of studied cryptosporidiosis in goat kids was found to be 5.84 percent in the Baghdad region, while [15] reported that the prevalence rate was 26.8 percent in sheep in various localities of the Ninevah province and that Basrah province had a distribution of positive Cryptosporidium infection among animal handlers and non-animal handlers.

Materials and methods

From September 2022 to the end of May 2023, 200 fecal samples from different places in Diyala province were taken from 100 instances of asymptomatic diarrhea in children, 65 lambs, and 35 goat kids, ranging in age from less than 3 months to more(lambs,kids) and less than one year to more (children). The study covered a variety of Diyala provincial locations ,and collected in plastic containers is in it potassium dichromate 2.5% and put in Ice box . By staining the swab with the Modified Ziel-Neelsen stain (MZN) [9] and floatation with Sheather's solution [16,17], each sample is examined microscopically to determine the presence of oocysts. ocular micrometre is used to measure oocyst length and breadth, The time to examined these samples 3 months. **Data Analysis:** The data were analyzed by SPSS program (2010), using Chi-square test(X^2) at ($P \leq 0.05$) level of significance.

Results

1. **Clinical examination : Clinically** according to (Table 4.1) from total number of (100: 65 lambs and 35 goat kids) about, (53lambs and 15 goat kids) exhibiting clinically watery diarrhea, anorexia, melancholy, abdominal discomfort, and weight loss due to a cryptosporidium infection.

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Figure 4-1: A locally breed goat kid less than 3 month old, with diarrhea infected by cryptosporidium spp.

Only 20 out of 100 instances involving children had cryptosporidium infection, some of which showed clinical signs of gastroenteritis, nausea, fever, vomiting, headaches, abdominal pains, and watery diarrhea.

Table 1: Species examined , number cases manifested clinically and infection rate y percentage infected by cryptosporidium

Species examined	No. examined	No. of cases manifested clinically	Infection rate %
Children	**100**	**21**	**21.0 %**
Lambs	**65**	**53**	**81.5 %**
Goat kids	**35**	**15**	**42.9 %**
Total	**200**	**89**	**45.5 %**
$X^2 = 58.504$, *P*-value= 0.041 S.D P<0.05			

2

. Microscopic examination of Fecal samples

For the purpose of determining the prevalence of infection with Cryptosporidium spp., 200 fecal samples were investigated using the traditional approach (flotation and staining with the modified Ziel-Neelsen (mZN) stain). revealed that in the Diyala area of Iraq, the total

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infection rate of Cryptosporidium spp. was 89/200 (45.5%).

2.1. Morphological characterization and measurements of Cryptosporidium spp. Oocysts

the microscopic review By floating Cryptosporidium parvum oocysts in a salt and sheathers solution, it was discovered that the oocysts were round or spherical with a thin membrane, and that the four sporozoites appeared as black entities inside the oocysts (Fig. 2). Ocular micromete was used to measure oocysts (Fig. 3). The average length and breadth measurements of C. parvum oocysts were almost 4.3 4.9 0.9m, and the oocysts stained with Modified Ziehl-Neelsen (m ZN) were spherical, dark red in colour, with a distinct halo around the oocysts. (Fig.4).

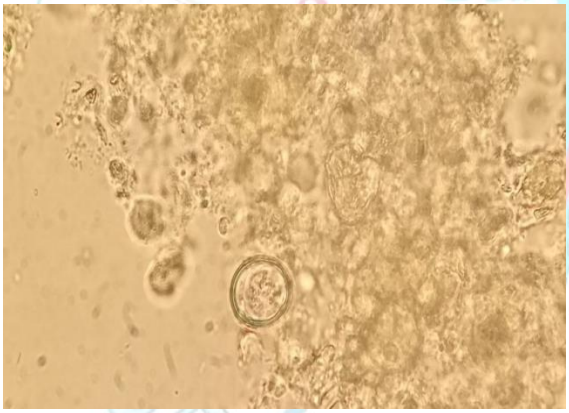

Figure (2): *C. parvum* oocysts, flotation with sheathers solution, appeared spherical or rounded with thin membrane (1000 x)

Figure (3): *C. parvum* oocysts flotation with salts solution measured 6.0×4.6 (1000 x)

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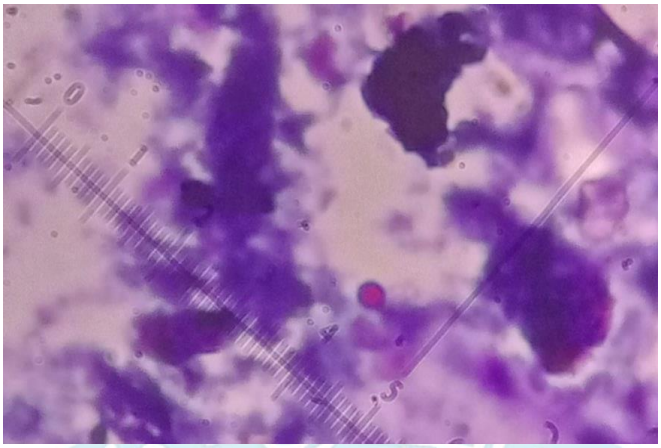

Figure (4):*C.parvum* oocysts stained with Modified Ziehl-Neelsen (400 x)

3. Epidemiological study : Distribution of infection rate according to sex, ages, area of study and months of collection samples.

3. 1. Infection rate of Cryptosporidium spp. infection in relation to sex

The relation of age with infection rate of *Cryptospordium* infection showed higher infection rate in male of lambs, kids and children than female as (Table 2).

Table 2: Infection rate and distribution of *Cryptospordium* according to the gender of children, lambs and kids .

Sex	Male N E - N P	Female N E - N P	Infection rate% Male - Female	Total N E - N P %
Children	51 - 15	49 - 6	29.4 - 12.2	100 - 21 21%
Lambs	55 - 40	20 - 13	72.7 - 65.0	65 - 53 81.5%
Kids	20 - 9	15 - 6	45.0 - 40.0	35 - 15 42.8%
X^2 = 14.105, *P*-value= 0.01495 S.D P<0.05				

3.2. Infection rate of Cryptosporidium spp. infection in relation to age groups.

The relation of age groups with infection rate of *Cryptosporidium* infection show high infection rate with

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significant difference (P<0.05) were recorded in the children at one year (A ranged from 1to 2 years) and the lowest infection rate 13.3% recorded at less than one years (Their ages ranged from 5 to 9 months) as (Table 3). Unfortunately, a high infection rate 66.7 % recorded at less than three months than more than three months of lambs (Table 4). Furthermore, the infection rate of *Cryptosporidium* infection in goat kids was higher in Less than three months 45% than more than three months (Table 5).

Table 3. Infection rate of Cryptospordium according to the age groups categories of Children

Age groups	N E. of fecal samples	N.P examined	Infection rate %
Less than one year	45	6	13.3%
One and more than one year	55	15	27.3%
Total	100	21	21.0%
$X^2 = 2.899$, *P*-value= 0.0886 N. S.D P<0.05			

Table 4. Infection rate of Cryptospordium according to the age groups categories of goat kids

Age groups	N E. of fecal samples	N.P examined	Infection rate %
Less than three months	20	9	45.0%
More than three months	15	6	40.0%
Total	35	15	42.9%
$X^2 = 0.088$, *P*-value= 0.766 N.S.D P<0.05			

Table 5. Infection rate of Cryptospordium according to the age groups categories of lambs

Age groups	N E. of fecal samples	N.P examined	Infection rate %
Less than & three months	30	20	66.7 %

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More than three months	**35**	**18**	**51.4 %**
Total			
X^2 = 1.545 , *P*-value= 0.2138 S.D P<0.05			

3.3. Infection rate of Cryptosporidium spp. in relation to areas of study.

According to the regional distribution of Cryptosporidium spp. infection rates in children, AlKhalis had a high infection rate of 30.0% and Khanaqin had a low infection rate of 10%. as in (Table 6)

Table 6: Show the infection rate of Cryptospordium in children according to area of study.

Area	**No. of Fecal sample from Children**	**No. of positive**	**Infection rate %**
Al Khalis	**30**	**9**	**30.0%**
Baqubah	**20**	**5**	**25.0%**
Bani saad	**15**	**3**	**20.0%**
Al Muqdadiya	**15**	**2**	**13.3%**
Baldruze	**10**	**1**	**10.0%**
Khanaqin	**10**	**1**	**10.0%**
Total	**100**	**21**	**21.0%**
X^2 = 3.657, *P*-value= 0.599 N. S.D P<0.05			

When the disease infection rate in lambs and young animals was reported, Al Khalis and Baqhuba had higher infection rates (80% compared to 50% in Baldruze and Khanaqin, respectively). as (Table 7).

Table 7: Show the infection rate of Cryptospordium in lambs and kids according to area of study.

Area	**No. of Fecal**	**No. of positive**	**Infection rate %**

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	sample from lambs and kids		
Al Khalis	**25**	**20**	**80.0%**
Baqubah	**15**	**12**	**80.0%**
Bani saad	**20**	**15**	**75.0 %**
Al Muqdadiya	**10**	**6**	**60.0%**
Baldruze	**20**	**10**	**50.0%**
Khanaqin	**10**	**5**	**50.0%**
Total	**100**	**68**	**68.0%**
X^2 = 7.858 , *P*-value= 0.164 N. S.D P<0.05			

3.4. Distribution of Cryptospordium spp. according to locality (Hose system) of study.

Regarding the area, the majority of infected children lived in the rural sector at a ratio of 27.7%, followed by the urban sector at a ratio of 13.04%. as (Table 8)

Table 8: Infection rate and distribution of Cryptospordium spp. according to the house system.

Locality	N E. of fecal samples	N.P examined	Infection rate %
Urban	**46**	**6**	**13. 0%**
Rural	**54**	**15**	**27.8 %**
Total	**100**	**21**	**21.0%**
X^2 = 3.251 , *P*-value= 0.0713 S.D P<0.05			

3.5. Infection rate of Cryptosporidium spp. in relation to months of study

The relationship between the study's months and the infection rate revealed that, in youngsters, lambs, and kids, respectively, the maximum infection was recorded in November at 33.3%, 62.5%, and 66.7%, while the lowest infection rate was recorded in May at 5%, 50%, and 33.3% with no statistically significant difference (P< 0.05).as (Table 9).

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Table (9) Infection rate of Cryptosporidium spp. in relation to months of study.

Year	Months	No. of samples Examined Children –Lambs –Kids	N.P.E Children –Lambs – Kids	Infection rate %
2022	Septemer	5- 5- 5	1- 2- 1	20% - 40% - 20%
	October	8- 5- 5	2- 2- 2	25% - 40% - 40%
	Novembe r	12- 8- 6	4 – 5- 4-	33.3%- 62.5%- 66.7%
	December	15- 10- 5	5 – 6- 2	33.3%- 60%- 40%
2023	January	10- 8- 5	3 – 5- 2	30% -62.5%- 40%
	February	10- 9- 2	2 – 4- 1	20%- 44.4%- 50%
	March	10- 6- 2	1- 3-1	10% - 50% - 50%
	April	10- 8- 2	2-4-1	20% - 50 % - 50%
2023	May	20- 6- 3	1 – 3- 1	5% - 50% - 33.3 - %
	Total	100- 65 - 35	21- 53- 15	21%- 81.5%- 42.9%
$X^2 = 9.152$, *P*-value= 0.329 N.S.D P<0.05				

Discussion

Cryptosporidiosis is considered to be a dangerous zoonotic infection brought on by a protozoan parasite with detrimental economic and public health repercussions on both humans and animals. **Clinically** lambs and kids of study Due to the nature of the Cryptospordium, which causes digestive issues and destroys the small intestine after infecting the digestive system and causing severe diarrhea, the study's symptoms of watery diarrhea, anorexia, sadness, abdominal discomfort, and weight loss may be explained [18,19,20,21]. Unfortunately, the intestinal protozoan Cryptospordium is frequently detected in cases of diarrheal sickness that affect people and other mammalian species across the world and causes diarrhea in children. [18, 20, 22]. Lambs and children in the current study may have received some

colostrum, either from their dams or synthetic colostrum, indicating that this may be associated to the varied immunocompetence status of individual animals. Clinical manifestations between individuals within groups are also present. Although it remained unknown what sort of colostrum each sheep produced.

The overall infection rate (45.5%) in children, lambs and kids in Diyala province can be explained by the fact that the fecal-oral route, which involves contaminated food and drink, is the primary means of transmission. According to [23,24], this can occur both directly and indirectly through contact with animal excrement and cross contamination, which occurs when hosts eat Cryptosporidium oocysts. Numerous transmission pathways, including person-to-person transmission, zoonotic transmission, food-borne transmission, and water-borne transmission, can cause cryptosporidium infections in humans [25].

Microscopically examination of Oocysts from *C. parvum* had 4.6 to 6.0 meters in length and width on average. These findings were comparable to those of Khalil (2010) [26], who reported that the average length and width measurements of *Cryptosporidium parvum* were (5.2 x 4.2) m. A *Cryptosporidium parvum* oocyst's average size was 5.01 × 4.56 m, according to Al-alousi and Mahmood's 2012 [27] research. Al-Zubaidi (2012) [8] evaluated the length and breadth of *C. parvum* oocysts in Iraq and found that they were, respectively, 5.2 0.41 m and 4.1 0.21 m.

Epidemiologically: Age and the prevalence of Cryptospordium infection were correlated, and male lambs, children, and young adults had greater rates of infection than females.

In the current study, The results of the geographical distribution of Cryptosporidium spp. infection rates in children indicated that Khanaqin had a 10% infection rate and AlKhalis had a 30% infection rate. When the disease infection rate in lambs and young animals was recorded as being higher in Al Khalis and Baqhuba (80% respectively) than in Baldruze and Khanaqin (50%) these findings were

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E-ISSN:2958-6178
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attributed to poor husbandry practices, contaminated water sources, drinking water, and the breeding of other domestic birds close to the farms.

These results are in line with studies conducted in Baghdad, Iraq, Iran, and Southern Egypt [24, 28, 29], which revealed that people who had contact with animals were more infectious than those who did not. Additionally, there was a strong relationship between the prevalence of Cryptosporidium and human contact with animals, with those who interacted with animals showing higher rates of infection than those who did not [24, 28, 29].

The correlation between age and infection rate was strong. Young ruminant animals frequently have infections with tiny age groups that are linked to Cryptosporidium, and children who are frequently exposed to animal excrement have an increased chance of developing the sickness., While their enhanced immunity accounts for the elderly population's lower infection rate. These findings were in agreement with those of previous investigators [18,19,23], who had noted that animals might acquire *Cryptosporidium* at a very early age, meaning they had been exposed to the parasite almost from the moment of birth. The majority of Cryptosporidium spp. infections occur in developing and emerging countries globally [6,8,23]. Lambs are known to be susceptible to *C. parvum* infection and to exhibit similar clinical symptoms and oocyst shedding patterns. Lambs are more petite. and easier to handle than calves, especially once they are 8 weeks old [8,23].

The clinical disease (diarrhea) and oocyst shedding in lambs with prior exposure (infected at 1 week of age) did not appear until they were 4 weeks old. were re-challenged at the age of 4 weeks, neither a clinical illness nor (detectable) oocyst shedding were present. Compared to animals, people have a lower risk of contracting cryptosporidiosis No age-related difference that was noteworthy existed [6,8,23].

According to months of research with cryptosporidiosis, there are various degrees of infection rate during the study's months. These variations may

be attributed to the climate's temperature and humidity, which have a role in infection rate. Additionally, the study did not cover every month of the year. The variation in the infection rate is influenced by each of those factors**.**

Conclusions: Lambs were more likely to contract cryptosporidiosis than goat kids and children. The infection rate of lambs, kids, and children was impacted by characteristics such sex, age groups, location of infection, and months of research.

Recommendation : Study prevalence of other intestinal protozoa infected Lambs, Kids. Increasing attention to environmental health, especially in rural areas. Furthermore, increasing the health awareness of parents about transmission
methods contributes effectively to reducing infection rates among children.

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