

The Prevalence of *Cryptosporidium* sp. in Patients of Some Basrah Hospitals Using Microscopy and Evaluation of Associated Epidemiological Factors.

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Abstract

In the current study, stool samples collected from patients at various hospitals in Basrah governorate such as Al-Fayhaa Teaching Hospital, Al-Zubair General Hospital, and Al-Sayyab Hospital. These samples were examined for infection with *Cryptosporidium* from December 1, 2024, to August 1, 2025.

The study was focused on detection of *Cryptosporidium* sp. In different areas of Basrah (Rural and Urban), gender, age groups and intensity of infection. A total of 193 stool samples were collected from people in Basrah governorate, of different ages, genders, and locations. The overall infection rate was 64.8%. Significant differences were mostly observed in the months of study. The infection rate was highest in March (76.9%) and lowest in December (37.1%), with a significant difference at $P \leq 0.009$. The highest infection rate was seen in rural areas, while the lowest was in urban areas, with a significant difference at $P \leq 0.009$.

The collected samples after staining were examined microscopically using modified Ziehl-Neelsen (MZN) stain for detection *Cryptosporidium* oocysts. The numbers and dimensions of the oocysts were recorded under the microscope and photographed using a camera mounted on a Leica microscope. The results were statistically analyzed using the Chi-square test at a probability level of $P \leq 0.05$.

Keywords: Prevalence – *Cryptosporidium* parasite – Microscopic examination – Environmental factors – Basrah, Iraq

المخلص

تضمنت الدراسة الحالية جمع و فحص عينات غائط من مرضى بعض مستشفيات محافظة البصرة شملت مستشفى الفحاء التعليمي ومستشفى الزبير الجمهوري ومستشفى السياب للفترة من 2024/12/1 لغاية 2025/8/1 للتأكد من إصابتها بطفيلي الأبواغ الخبيثة *Cryptosporidium*. تم جمع 193 عينة غائط من مختلف الأعمار و من كلا الجنسين ومن مناطق مختلفة من محافظة البصرة. بلغت نسبة الإصابة الكلية 64.8%. سجلت فروقات معنوية بين أشهر الدراسة المختلفة حيث بلغت أعلى نسبة مئوية للإصابة 76.9% في شهر آذار وأقلها 37.1% في شهر كانون الأول وبفارق معنوي $P \leq 0.009$. بلغت أعلى نسبة مئوية للإصابة في المناطق الريفية وأقلها في المناطق الحضرية وبفارق معنوي $P \leq 0.009$.

تم فحص العينات التي تم جمعها مباشرة مجهرياً بعد تصبغها بصيغة زيل نيلسون المحورة Modified Ziehl Neilsen للتحري عن أكياس بيض الطفيلي. سجلت أعداد وأبعاد أكياس البيض في حقل المجهر وصورت باستعمال كاميرا مثبتة على متن مجهر نوع Leica. تم تحليل النتائج احصائياً باستعمال مربع كاي وتحت مستوى احتمالية $P \leq 0.05$.

الكلمات المفتاحية: نسبة الانتشار - طفيلي الأبواغ الخبيثة - فحص مجهري - العوامل البيئية - بصرة العراق

Introduction

Cryptosporidium infects human and a wide range of animals, particularly cattle, mammals, fish, and birds (Ghazy, 2015; Costa *et al.*, 2020; Ryan *et al.*, 2021). Infection mainly affects individuals with weak immune systems, causing *cryptosporidiosis*, which is considered a zoonotic parasitic disease. It is more common

among children suffering from malnutrition (Khan *et al.*, 2004 Caccio, 2005).

Cryptosporidium belongs to the phylum Apicomplexa (Bouazid, 2018). Infection with *Cryptosporidium* leads to acute and chronic diarrhea in humans (Abdulsadah *et al.*, 2013; Khalil, 2018) and is widespread among immunodeficient individuals. It causes

persistent diarrhea that can lead to malnutrition and growth retardation in children (Huang *et al.*, 2004, Haider *et al.*, 2014).

The parasite is easily transmitted among children in nurseries, schools, and kindergartens. Xiao and Ryan (2008) indicated that *Cryptosporidium parvum* and *Cryptosporidium hominis* account for about 90% of human infections in most regions of the world, although several species of the parasite can infect human.

There are many laboratories diagnostic methods used to detect the parasite, and the modified Ziehl–Neelsen (MZN) stain is considered the best and most successful method for diagnosis (Gunho *et al.*, 2019).

Transmission occurs through contaminated water and food containing the parasite's oocysts (Keusch *et al.*, 2010; Zakir *et al.*, 2021), as well as through contact with infected individuals and animals (Hassan *et al.*, 2011). The oocysts are highly resistant to many disinfectants (Gabri *et al.*, 2010), Sterilizers and water treatment processes (Guy *et al.*, 2021; Awad and Naser, 2014; Shaltout, 2000).

Sewage water, heavy rainfall runoff, water from slaughterhouses located near rivers, and animal manure (Caffarena *et al.*, 2020) in agricultural fields are among the main sources of *Cryptosporidium* oocysts in water (Graczyk *et al.*, 2008). The oocysts can survive in water for extended periods—up to nearly a full year.

According to the seriousness of the disease in humans and the limited number of studies on this parasite in Basrah Governorate (Awad & Naser, 2014; Mahdi, 1996; Jassim, 2002), this present study was conducted to determine the prevalence of the parasite and evaluate the impact of some Basrah epidemiological factors in selected patients.

Materials and Methods

A total of 193 stool samples were collected from patients in several hospitals in Basrah Governorate, including Al-Zubair General Hospital, Al-Sayyab Hospital, and Al-Fayhaa Teaching Hospital, during the period from December 1, 2024, to August 1, 2025. The samples were collected in airtight plastic containers and transported immediately to the Parasitology Laboratory, Department of Biology, College of Education for Pure Sciences.

During sample collection, a questionnaire form was distributed, which included the patient's name, gender, age, area of residence. Each stool sample was used for microscopic examination (Double – blind) to detect the parasite using the modified Ziehl–Neelsen (MZN) staining method.

For microscopic examination, a smear was prepared from each stool sample by mixing a small amount of feces, approximately the size of a matchstick head, on a clean glass slide with a drop of distilled water. The smear spread evenly over the entire surface of the slide and allowed air to dry.

After drying, concentrated Carbol Fuchsin (red stain) was applied and left for three minutes. The slide was then rinsed with tap water, decolorized with 3% acid alcohol for 30 seconds, washed again with water, and air-dried. It was then counterstained with methylene blue for two minutes, washed with tap water, and allowed to dry completely.

The stained slides were examined under a light microscope at 100× and 40× magnification using immersion oil to detect oocysts of the parasite. The number and dimensions of the oocysts observed in each microscopic field were recorded, and photomicrographs were taken using a camera mounted on the microscope.

Statistical Analysis

The data of the present study were analyzed using the Chi-square (χ^2) test to assess the association between *Cryptosporidium* infection and the months of the study period, age, sex, and area of residence (rural vs. urban). All statistical tests were performed at a significance level of $P < 0.05$.

Results and Discussion

The oocysts of *Cryptosporidium* stained with modified Ziehl–Neelsen stain appeared oval in shape and red in color (Lamison, 2016; Khurana and Ghaudhary, 2018; Khan, 2019). The mean dimensions of the oocysts were $4 \times 5.2 \mu\text{m}$ (Figure 1).

The results of the present study showed that the highest prevalence of *Cryptosporidium* infection was recorded in March (76.9%), while the lowest prevalence was observed in December (37.1%), with a statistically significant difference ($P \leq 0.009$), table (1) and figure (2).. The higher infection rate in March may be attributed to favorable climatic conditions, particularly suitable temperature and humidity, which promote the development of *Cryptosporidium* oocysts and enhance their resistance and survival in the environment for longer periods.

The findings of the current study are in agreement with those of Raad (2019) in Al-Najaf Al-Ashraf Province, who reported the highest infection rate in February and the lowest in January. However, they differ from the results of Ahmed *et al.* (2012) in Babil Province, who found that the highest prevalence of cryptosporidiosis was recorded in September (33.7%) and the lowest in January (4.5%).

The highest infection rate was recorded in the age group 21–30 years, while the lowest rate ($\leq 10\%$) was observed in the youngest age group. No statistically significant differences

were found among the age groups under study, table (2) and figure (3). This may be attributed to the fact that all age groups are similarly exposed to the risk of infection with the parasite through contaminated hands, consumption of fruits and vegetables, and drinking water contaminated with *Cryptosporidium* oocysts (Kadim, 2014; Ahmed and Jasim, 2023), Monsef *et al.* (2008) indicated that the highest infection rate was found at (40 – 60) year in Iran.

Khalil (2000) in Nineveh Province, however, reported significant differences in infection rates among age groups.

The highest infection rate was recorded in males (65%), while the lowest rate was observed in females (64%), and no statistically significant difference was found between the two sexes, table (3) and figure (4). Some studies indicate that there are no significant differences between the two sexes (Casemore, 1990), despite the slightly higher infection rate in males. This may be attributed to the greater activity of males and their more frequent direct contact with the external environment and farm animals compared to females.

The findings of the present study are in agreement with those reported by Al-Samarrie (1999) in Tikrit City, as well as by Al-Kilani (1998) and Al-Kabasi *et al.* (2014), in Baghdad, who both indicated that the prevalence of the disease was higher in males than in females. Similarly, Nesrin (2008) in Sulaymaniyah reported a higher infection rate in males than in females, and comparable results were observed in the studies of Al-Mamiouri (2000) and Oleiwi (2015) in Babil Province, as well as in the study of Akram and Besaad (2020) in Al-Rifai District, Thi Qar Province. On the other side Saneian *et al.* (2010) from Iran mentioned that the infection in female was higher than male.

The highest infection rate in the present study was recorded in rural areas, reaching

during May 100%, while the lowest rate was observed in urban areas (30.8%) during December, with a statistically significant difference ($P \leq 0.000$), table (4) and figure (5). The findings indicate that *Cryptosporidium* is more prevalent in rural areas than in urban ones. This higher prevalence in rural regions may be attributed to the fact that most infected individuals belong to low-income families who are continuously exposed to *Cryptosporidium* oocysts through fecal contamination, lack of access to safe drinking water, insufficient sanitary services, direct contact with animals, and the use of animal feces as fertilizer for field crops. In addition, low educational and health awareness levels in these families may contribute to the increased infection rates (Othman, 2000; Al-Yassaree, 2004; Goh *et al.*, 2004; ;Al-Hadithi & Awad, 2015; Mulla, 2008)

These results agree with Al-Kubaisi (2014) in Baghdad City but differ from Mohammed (2018) in Al-Diwaniyah, who reported a 63.3% infection rate in rural areas. Ghanadi *et al.* (2022) showed that the highest infection rate was in urban area of Iran than rural area.

The highest number of *Cryptosporidium* oocysts per microscopic field was observed in group four (++++) with 50 oocysts, while the lowest number was recorded in group one (+) with a single oocyst. A statistically significant difference was found between the groups ($P \leq 0.000024$), table (5) and figure (6).

Conclusion:

The current study shows that cryptosporidiosis is a common infection in Basrah Province causing diarrhea in patients infected with a parasite. The study includes examination of 193 fecal samples collected from different hospitals in Basrah with 64.8% total percentage infection rate.

The infection rate in male patients was higher than in female, but without statistically

significant difference between them. The parasite is more prevalent in age group (21 – 30) years, and more common in rural than urban areas with high significant differences.

The current study showed that the modified Ziehl-Neelsen stain is a better dye used for staining the parasite. The Health Department should provide pure water for people in Basrah.

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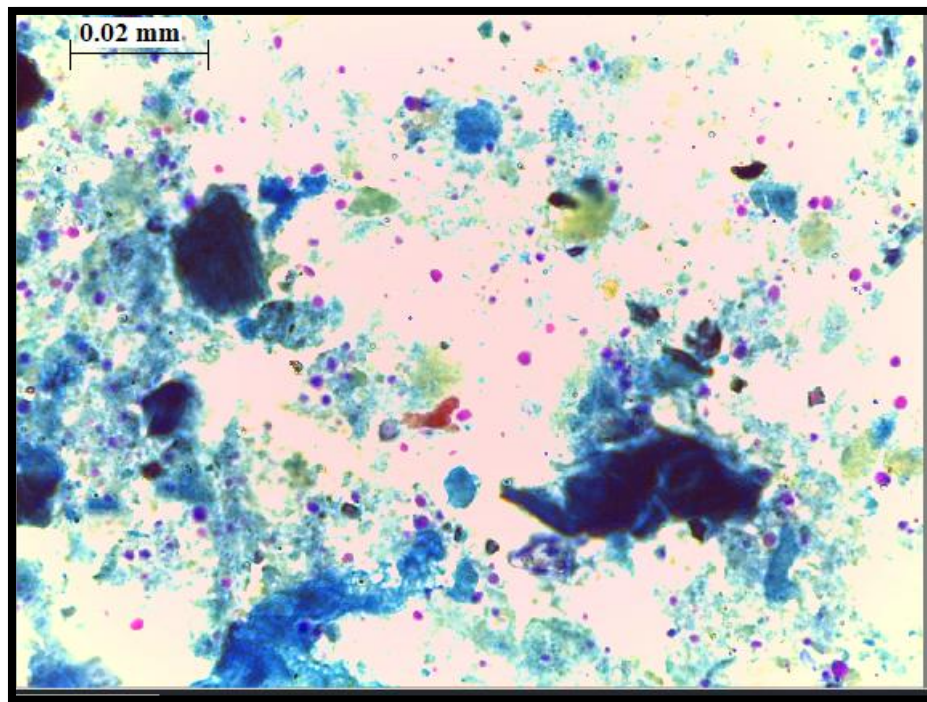


Figure (1): Oocyst of *Cryptosporidium* in the feces of one of the infected patients stained with modified Ziehl–Neelsen stain.

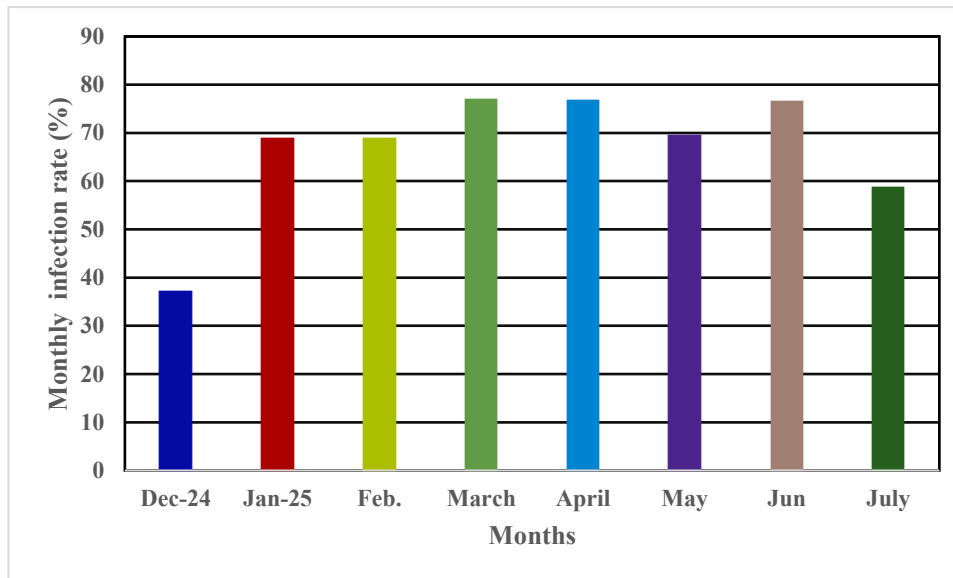


Figure (2): Monthly Prevalence of *Cryptosporidium* Infection among Hospitalized Patients

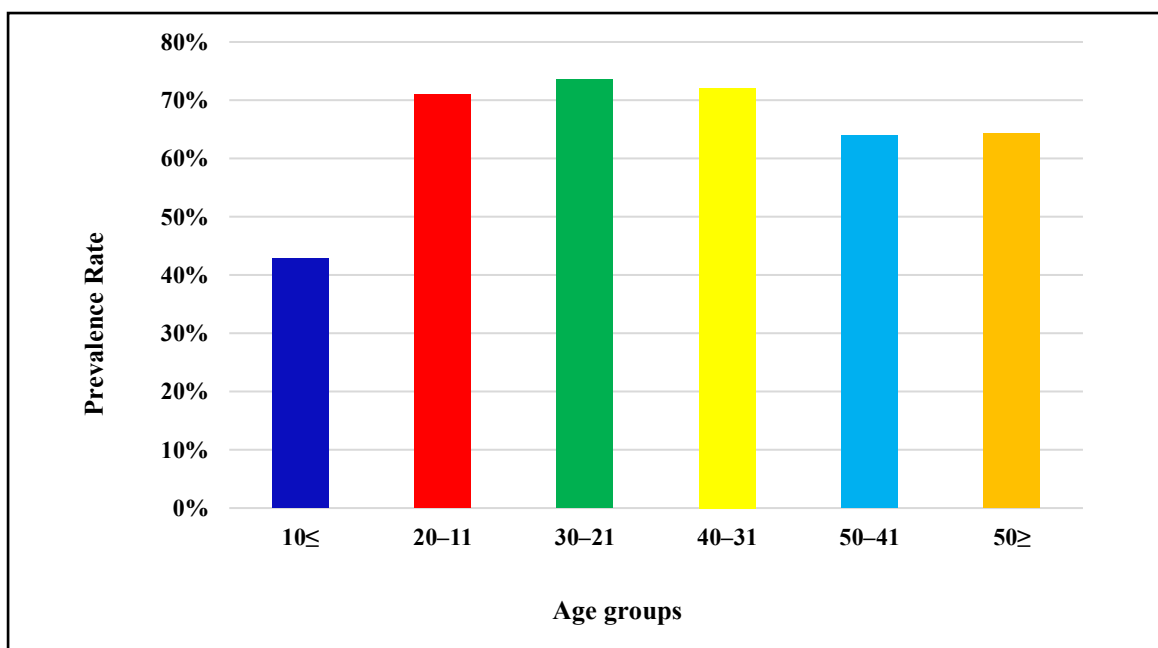


Figure (3): Prevalence of *Cryptosporidium* infection according to age groups

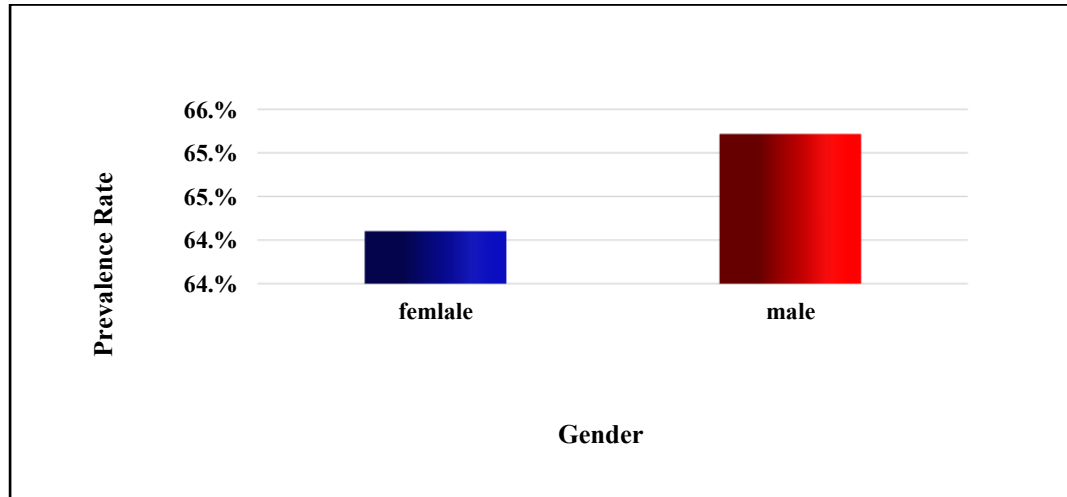


Figure (4): Prevalence of *Cryptosporidium* infection according to the gender of infected individuals

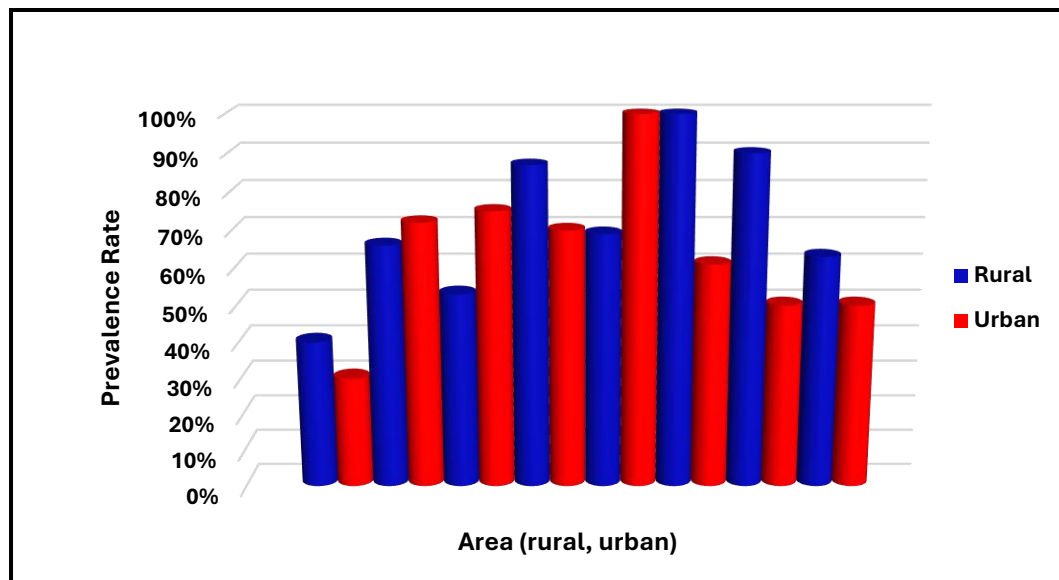


Figure (5): Severity of *Cryptosporidium* infection according to the number of oocysts per microscopic field

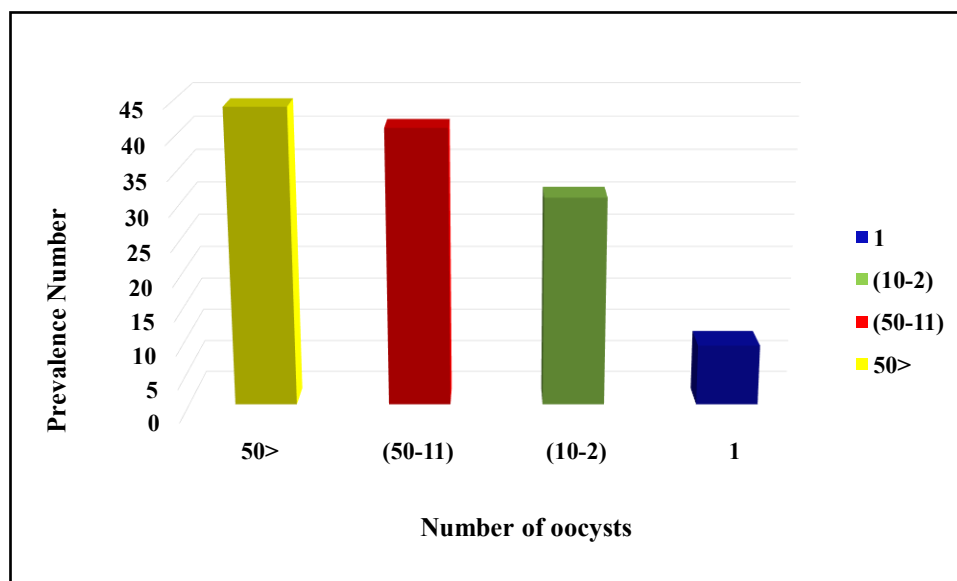


Figure (6): Severity of *Cryptosporidium* infection according to the number of oocysts per microscopic field

Table (1): Monthly Prevalence of *Cryptosporidium* Infection among Hospitalized Patients

Month	Examined Samples	Infected Samples	Percentage (%)
December 2024	35	13	37.142
January 2025	29	20	68.965
February	29	20	68.965
March	26	20	76.923
April	17	13	76.740
May	23	16	69.565
June	17	13	76.470
July	17	10	58.823
Total	193	125	64.766

$$\chi^2=18.640 \quad df=7 \quad p \leq 0.009$$

Table (2): Prevalence of *Cryptosporidium* Infection According to Age Groups

Age groups	Examined Samples	Infected Samples	Percentage (%)
≥ 10	35	15	% 42.857
11-20	31	22	% 70.967
21-30	34	25	% 73.529
31-40	43	31	% 72.093
41-50	36	23	% 63.888
≤ 50	14	9	% 64.285
Total	193	125	% 64.766

$$\chi^2=10.072 \quad df=6 \quad p \leq 0.073$$

Table (3): Prevalence of *Cryptosporidium* infection according to the gender of infected individuals

Gender	Examined Samples	Infected Samples	Percentage (%)
Male	115	75	% 65.217
Female	78	50	% 64.102
Total	193	125	% 64.766

$$\chi^2=0.008 \quad df=1 \quad p \leq 0.930$$

Table (4): Prevalence of *Cryptosporidium* infection according to area (rural, urban)

Months	Area	Examined Samples	Infected Samples	Percentage (%)
December	Rural	22	9	40.9090
	Urban	13	4	30.769
January	Rural	18	12	66.66
	Urban	11	8	72.72
February	Rural	13	7	53.84
	Urban	16	12	75.0
March	Rural	16	14	87.5
	Urban	10	7	70.0
April	Rural	13	9	69.23
	Urban	4	4	100.0

May	Rural	5	5	100.0
	Urban	18	11	61.11
June	Rural	11	10	90.90
	Urban	6	3	
July	Rural	11	7	63.6
	Urban	6	3	50.0
Total		193	125	64.766

$$\chi^2 = 265.7 \quad df=14 \quad p \leq 0.000$$

Table (5): Intensity of *Cryptosporidium* infection according to the number of oocysts per microscopic field (microscopy).

Number of Oocysts	Number of Infected Individuals
+	9
++ (2–10 oocysts)	31
+++ (11–50 oocysts)	41
++++ (more than 50 oocysts)	44
Total	125

$$\chi^2 = 24.088 \quad df=3 \quad p \leq 0.000024$$