Diagnosis of Cryptosporidium parvum in human and calves in Tikrit city and its surroundings

[1]Al-khaizuran Al-muhalab Khalaf, [2]Omaima Ibrahim Mahmood

[1,2]Microbiology, Veterinary Collage, University of Tikrit, Tikrit, Iraq

aa230003pve@st.tu.edu.iq

dr.omaimapara@tu.edu.iq

Abstract:

Background: *Cryptosporidium parvum* is a coccidian parasite capable of infecting several mammals, including humans. This parasite significantly contributes to newborn diarrhea and morbidity in calves, resulting in considerable economic losses in the livestock industry. This study was conducted to determine the prevalence of *C.parvum* in both humans and calves using three diagnostic methods: flotation, cold and hot Modified Ziehl-Neelsen staining. A total of 200 stool samples from humans and 200 fecal samples from calves were collected. The sample collection was carried out between September 26, 2024, and June 2, 2025m by using flotation, cold and hot Modified Ziehl-Neelsen staining methods. The results revealed that the number of oocysts detected in calves was comparable to that found in humans, with prevalence rates of 3.5% and 6% respectively, using the flotation method. In humans and calves, diagnosis using Modified Ziehl-Neelsen staining, both hot and cold methods, demonstrated slightly varying results. The cold MZN technique showed a higher detection rate (8.5% and 5%) compared to the hot method (8% and 3%) respectively, indicating a potential sensitivity difference between the two techniques. Regarding demographic distribution, *C. parvum* infection in both human and calves was recorded in both males and females at similar rates, with males showing a slightly higher infection rate (5.5% and 3%)respectively, compared to females (3.5% and 2%) respectively. Age-based analysis indicated that children aged 1–6 years had the highest infection rate (4.5%), whereas the lowest was observed in the 4–6 year age group (0.5%). In calves, the infection was detected across all age groups with no statistically significant differences ($p > 0.05$).

Keywords: *Cryptosporidium parvum*, human, calves,flotation methods, Modified Ziehl-Neelsen staining.

تشخيص *Cryptosporidium parvum* في الانسان والعجول في مدينة تكريت وضواحيها

الخيزران المهلب خلف،[1] أميمة إبراهيم محمود[2]

[1,2] علم الأحياء المجهرية، كلية الطب البيطري، جامعة تكريت، تكريت، العراق

aa230003pve@st.tu.edu.iq

dr.omaimapara@tu.edu.iq

يُعد Cryptosporidium parvum ذوات المعقد القمي Apicomplexa من فصيلة الكوكسيديا قادرًا على إصابة نطاق واسع من الثدييات، بما في ذلك الإنسان. ويُعتبر أحد أهم العوامل المسببة للإسهال الوليدي والاعتلال الصحي في العجول، مما يؤدي إلى خسائر اقتصادية كبيرة في صناعة الثروة الحيوانية. هدفت هذه الدراسة إلى تحديد معدل انتشار C. parvum لدى كل من البشر والعجول باستخدام ثلاث طرق تشخيصية: الطفو، وصبغة زيل-نيلسن المعدلة (MZN) بنوعيها الساخن والبارد. تم جمع 200 عينة براز من البشر و200 عينة براز من العجول بين 26 أيلول 2024 و2 حزيران 2025. أظهرت نتائج طريقة الطفو أن معدل انتشار الأكياس البيضية (oocysts) بلغ 6% عند البشر و3.5% عند العجول. أما التشخيص باستخدام صبغة MZN فقد أظهر اختلافات طفيفة في نسب الكشف؛ إذ أظهرت التقنية الباردة حساسية أعلى (8.5% لدى البشر و5% لدى العجول) مقارنة بالتقنية الساخنة (8% و3% على التوالي). وفيما يتعلق بالعوامل الديموغرافية، سُجِّلت الإصابات في كلا الجنسين مع ارتفاع طفيف عند الذكور (5.5% لدى البشر و3% لدى العجول) مقارنة بالإناث (3.5% و2% على التوالي). وأوضحت التحاليل العمرية أن الأطفال الذين تتراوح أعمارهم بين 1–6 سنوات كانوا الأكثر إصابة (4.5%)، بينما سُجِّلت أقل نسبة في الفئة العمرية 4–6 سنوات (0.5%). أما في العجول، فقد لوحظت الإصابة في جميع الفئات العمرية دون فروق ذات دلالة إحصائية ($p > 0.05$).

الكلمات المفتاحية: الابواغ الخبيثة ، الإنسان، العجول، طرق التعويم، صبغة زيل-نيلسن المعدلة.

1. Introduction

Cryptosporidiosis, a neglected zoonotic disease, is transmitted through interactions between humans and household animals or nearby wildlife reservoirs[1]. Cryptosporidium spp. infects humans and several animals, leading to Cryptosporidiosis. Cryptosporidiosis in mammals is a notable infectious diarrheal disease affecting young calves and humans of all ages [2]. The parasite completes

its life cycle in one host, alternating between asexual and sexual reproduction. Cryptosporidium parvum is a global zoonotic pathogen. Parasite reproduction in intestinal epithelial cells (IECs) produces oocysts that spread infection via the feces-oral pathway. Cryptosporidium, a tiny protozoan parasite, infects vertebrates' digestive and respiratory epithelial cells' microvillous regions [4,5].

Intestinal parasite infection is one of the most important problems that human societies suffer from, and one of the risks that threaten public health in developing countries, especially among children, as they cause diarrhea, malnutrition, and delayed physical and mental growth. Cryptosporidiasis is one of the opportunistic intestinal diseases that cause diarrhea in the world, especially among children with immunocompromised deficiency, while it is asymptomatic in immunocompetent people [5].

Calf diarrhoea is a diarrheal disorder that mostly affects calves in their first days [6]. Diarrhoea renders calves more susceptible to infections, especially bovine respiratory illness, and slows growth and performance during lactation [7]. Infected animals excrete C. parvum oocysts, spreading illness. Millions of oocysts spread in the first two weeks after infection, indicating infection in feces[8,9].Half of dairy calves aged 1–3 weeks actively discharge oocysts. [10]. The present study is to examine the clinical evaluation of wet mount preparations compared to modifications of Ziehl–Neelsen staining for the diagnosis of *C. parvum* in children under six years of age in Tikrit province.

2. Materials and methods:

Samples collection

Stool samples were obtained from 200 human feces and 200 calf feces. Sample collection occurred from September 26, 2024, to June 2, 2025. The inclusion

criterion was diarrhea, defined as the passage of three or more loose or watery stools per day, or at a frequency exceeding the individual's norm.

Fecal samples were collected from children attending and inpatients from government and private hospitals in Salah al-Din Governorate (Tikrit Teaching Hospital, Baiji General Hospital, Al-Dhuluiya General Hospital). The samples were collected from children aged between 1 day and 6 years. Each sample was obtained using a clean, sealed 40 ml plastic container. Relevant patient information was recorded, including name, age, gender, weight, and symptoms such as diarrhea, weight loss, fever, loss of appetite, and signs of gastroenteritis. Each sample was assigned a unique number before being sent to the laboratory for testing.

Fecal samples were collected from both sexes of calves ranging in age from two months to two years. The samples were obtained from local fields and farms in Salah al-Din Governorate (Tikrit, Al-Alam, Al-Dhuluiya, Yathrib, and other areas)and taken directly from the animals' rectums, ensuring that medical gloves were worn with the assistance of a specialist. Clinical signs of each animal were recorded, including severe watery diarrhea, moderate diarrhea, fever, emaciation, and recumbency. Samples were collected in clean, sealed plastic containers labeled with the animal’s identification number, sex, and age. They were then transferred to the laboratory for testing.

Microscopic Examination

The microscopic examination of the sample involved two methods: the flotation method using a sugar solution and the Modified Ziehl–Neelsen stained method.

A- Flotation method

The principle of flotation test is based on converting the density of the solution to a density higher than that of the parasites, which leads to an increase in the concentration of oocysts. [11]

B- Staining Techniques :

Cold method of Modified Ziehl–Neelsen (MZN) staining for Fecal Smears: This method dons as mentioned by [11,12].

Hot method of Modified Ziehl–Neelsen (MZN) staining of fecal smears : The technique done by fixing it throgh passing the glass slide over a flame using a gas burner[12].

Statistical analysis

Statistical analysis: All collected data were organized into tables. Pearson Chi-square (X^2) was employed as a test of significance. Differences were deemed significant when the probability (P) was less than 0.05 or 0.01.

3. Results

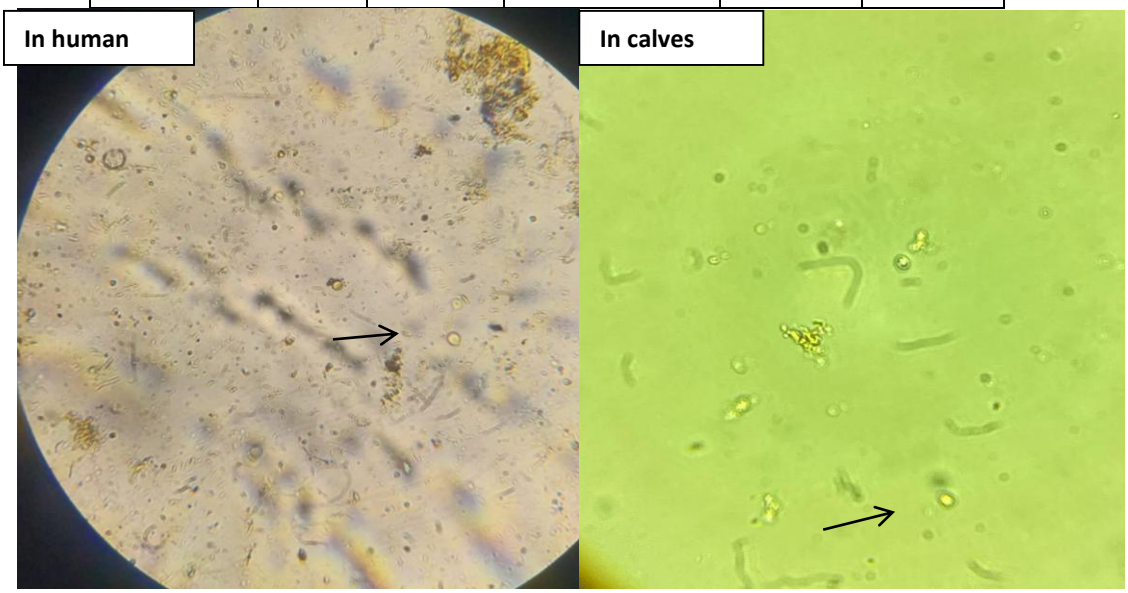
The results of the current study showed that the number of oocysts detected in calves was close to that detected in humans using the flotation method, with 7 cases (3.5%) in calves and 12 cases (6%) in humans, respectively.

The oocyst appeared spherical and transparent, with dark walls containing dark spots represented by spores and a large gap, as in the Table (1), Figure (1).

Table (1): Concentrated sugar solution circulating

	No	Positive result	Percentage %	P-value	Chi^2

Human Samples	200	12	6%	0.225	1.471
Claves Samples	200	7	3.5%		



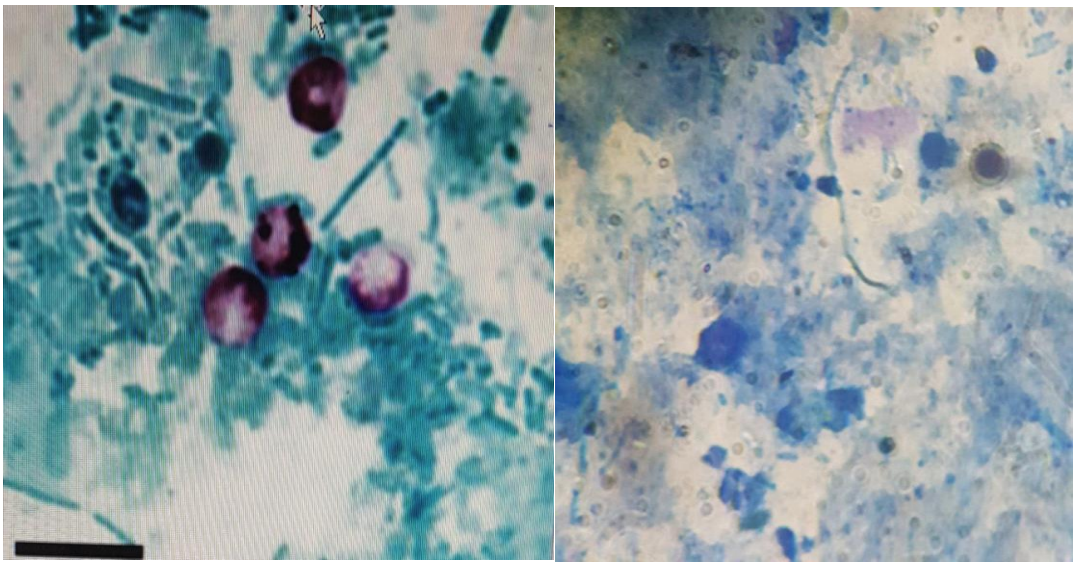
Figure(1): Oocysts of the *C. pavum* isolated from human and calves feces by flotation method, magnification 100X.

The current study demonstrated the percentage of human infection with Cryptosporidium parvum using both hot and cold Ziehl-Neelsen (ZN) staining methods. The cold ZN method showed 17 positive results (8.5%), while the hot ZN method showed 16 positive results (8%).

Oocysts of the parasite appeared after being stained with modified Ziehl-Neelsen staining, with a spherical shape and a reddish-purple colour, while the rest of the feces appeared blue, as shown in Figure (2).

Table (2): Oocysts of the *C. pavum* isolated from human feces by hot and cold ZN.

Human Samples	No	Methods	Positive result	Percentage %	P-value	Chi^2
	200	ZN staining Cold	17	8.5%	0.862	0.030
	200	ZN staining Hot	16	8%		

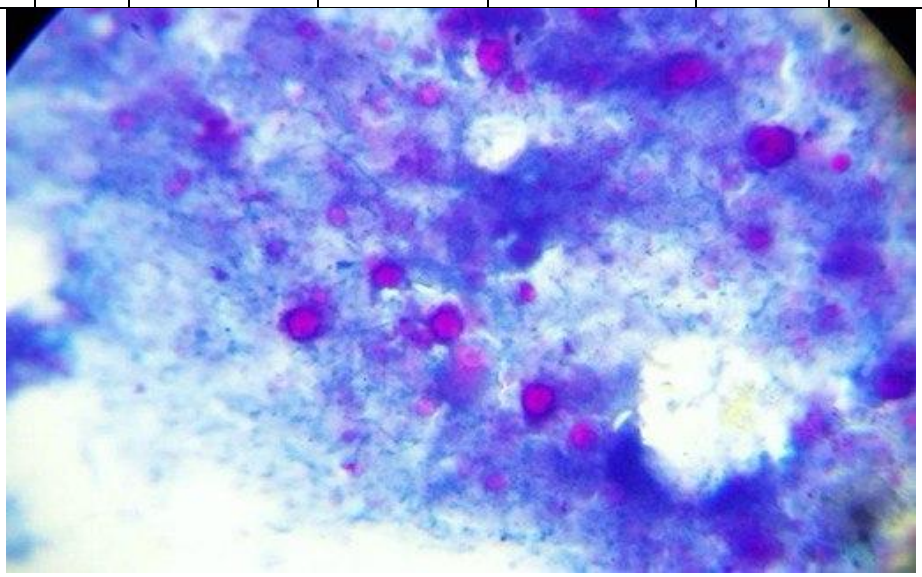


Figure(2): Oocysts of the *C.pavum* isolated from human feces by hot and cold ZN, magnification 100X.

The current study demonstrated the percentage of human infection with *C.pavum* by using hot and cold MZN. The cold method showed 10 positive result with 5% percent, while hot ZN showed 6 positive result with 3 % percent.As shown in Table (3) and Figure(3).

Table(3):Oocysts of the *C.pavum* isolated from calves feces by hot and cold ZN.

Claves Samples	**No**		**Positive result**	**Percentage %**	**P-value**	**Chi^2**
	200	ZN staining Cold	10	5%	0.317	1.0
	200	ZN staining Hot	6	3%		



Figure(3): Oocysts of the *C.pavum* isolated from human feces by hot and cold ZN, magnification 100X.

The results showed that C. parvum infects both sexes in humans at relatively similar rates. The infection rate among males was 11 cases (5.5%), while the infection rate among females was 7 cases (3.5%).

Table (4): *C.pavum* prevalence by sex group of human based on Ziehl-Neelsen.

Sex	**Total number**	**Positive result**	**Percentage %**	Chi^2	p-value
Male	**118**	**11**	**5.5%**	0.889	0.346
Female	**82**	**7**	**3.5%**		

The current results showed that the Cryptosporidium parasite infects all age groups at different rates and in both sexes. the highest infection rate among age groups was among children aged 1-6 years, reaching 4.5%, and the lowest infection rate was among age groups 4-6 years, reaching 0.5%.

Table (5): *C.parvum* prevalence by age group of human based on Ziehl-Neelsen.

Age groups	**Total number**	**Positive result**	**Percentage %**	Chi^2	p-value
1-6 months	115	9	4.5%	9.111	0.028
7-12 months	38	6	3%		
1-3 years	28	2	1%		
4-6 years	19	1	0.5%		
Total	200	18	9%		

The data indicated that *C.parvum* infects both sexes at comparable rates. The study indicated that the infection rate among males was 6 (3%), while the infection rate among females was 4 (2%).

Table (6): *C.parvum* prevalence by sex group of calves based on Ziehl-Neelsen.

Sex	**Total number**	**Positive result**	**Percentage %**	**Chi^2**	**p-value**
Male	126	6	3%	0.400	0.527
Female	74	4	2%		
Total	200	10	5%		

The current results showed that the *C.parvum* infects all age groups in calves. The study showed no significant differences in the infection rate among age groups in calves at p-value > 0.05.

Table (7): *C. parvum* prevalence by age group of calves based on Ziehl-Neelsen

Age groups	**Total number**	**Positive result**	**Percentage %**	Chi^2	p-value
1-5 weeks	**67**	**4**	**2%**	2.00	0.572
6 weeks -3 months	**26**	**2**	**1%**		
4-5 years	**13**	**1**	**0.5%**		
1- >5 years	**94**	**3**	**1.5%**		
Total	**200**	**10**	**5%**		

4. Discussion

The spread of intestinal parasites, including the Cryptosporium parasite, among the population is a major health problem that is being investigated through surveys in hospitals, cities, and schools. These parasites are widespread among all age groups and in all urban and rural areas, which provide a suitable environment for the transmission of these parasites[13]. The high transmission and infection rate of these parasites is attributed to the ease with which the infectious stages of these parasites can be transmitted from one person to another through contaminated water or food. Sources of exposure to these parasites vary depending on health culture, social customs, the way of coexisting with animals, and the type of nutrition. Infection with some parasites also increases due to what is known as opportunistic infection, especially when the immune status of the infected person is low, especially in patients receiving chemotherapy and AIDS patients[13].

The incidence of Cryptosporidiosis recorded in the current study is lower than that recorded in many previous studies in Iraq such as the study by [14] in Babylon Governorate where the incidence of the parasite reached 44.1%, the study by[15] in Basra Governorate where the incidence of the parasite reached 16.79%. While the infection rate with the cryptosporidiosis parasite recorded in the current study is higher than the rate recorded in some studies as[16] in Diyala Governorate by 2.8%.

The modified acid-fast staining technique is a widely recognized method for the detection of Cryptosporidium oocysts in fecal samples. Due to its high sensitivity and specificity, it is routinely employed in clinical diagnostic laboratories, particularly in cases of low parasite burden. Furthermore, this method offers practical advantages, such as speed and ease of use, and does not require advanced technical skills[17].

The current study noted variations in prevalence rates among age groups, with the highest incidence of cryptosporidiosis observed in children under 6 months of age. The frequency of Cryptosporidium in children under five years old was 8.2%, and 14.3% in Indian children aged six months to one year[18]. Milk bottles or non-breastfeeders crawling on contaminated surfaces may cause this. Though hypothetical, reduced immune capabilities may cause cryptosporidiosis from low-dose infections, and recurrent low-dose infections may protect older children from Cryptosporidium[19]. This study confirmed [19]'s Babylon Governorate infection rate of 2.6% males and 1.1% females. A Palestine study found 11.3% male and 9.7% female infection rates[20]. Cryptosporium parasite infection rates in Isfahan[21], Iran, were 2% male and 2.6% female. The current study contradicts [22], which found that Qadisiyah Governorate females (56.6%) had a higher infection rate than males (43.3%).

This study in calves focuses on the detection of oocysts using flotation and MZN staining using both hot and cold procedures. The largest percentage was observed in cold procedures, followed by hot methods, and finally flotation methods. In the research conducted by Mahami et al., 3.6% (8/227) of the examined cattle were infected with Cryptosporidium spp.[23]. Various factors, including breed, husbandry and management systems, age, nursing circumstances of calves, seasonal variations, and other elements, can account for the disparities in the frequency of Cryptosporidium spp. among calves in different global regions[24].

5. Conclusion

The study concludes that *C.parvum* is a zoonotic parasite capable of infecting both humans and calves with similar morphological characteristics of oocysts. Cold MZN staining proved slightly more effective in detecting the parasite in human samples. Infection occurs across all age groups and both sexes, with higher prevalence in

young children and no significant age-related differences in calves. These findings highlight the importance of routine screening and effective diagnostic methods to detect and manage Cryptosporidium infections in both human and animal populations.

6. References

1. Enbom, T., Suominen, K., Laitinen, S., Ollgren, J., Autio, T., & Rimhanen-Finne, R. J. A. V. S. Cryptosporidium parvum: an emerging occupational zoonosis in Finland.2023. *65*(1), 25.
2. Pumipuntu, N., & Piratae, S. J. V. w. Cryptosporidiosis: A zoonotic disease concern. 2018. *11*(5), 681.
3. Çelik, Ö., Aslan Çelik, B., Ayan, A., Orunç Kılınç, Ö., Ercan, K., Selçuk, M., . . . Oktay Ayan, Ö. J. E. J. o. V. S. Microscopic and Molecular Prevalence of Cryptosporidium spp. in Lambs in Siirt, Turkey.2023 *54*(5).
4. Gerace, E., Presti, V. D. M. L., Biondo, C. J. E. J. o. M., & Immunology. Cryptosporidium infection: epidemiology, pathogenesis, and differential diagnosis.2019; *9*(4), 119-123.
5. Costa, D., Razakandrainibe, R., Valot, S., Vannier, M., Sautour, M., Basmaciyan, L., Ballet, J.-J. J. M. Epidemiology of Cryptosporidiosis in France from 2017 to 2019. *2020; 8*(9), 1358.
6. Suler, D., Mullins, D., Rudge, T., & Ashurst, J. J. N. A. j. o. m. s. Cryptosporidium parvum infection following contact with livestock.2016. *8*(7), 323.
7. Uga, S., Matsuo, J., Kono, E., Kimura, K., Inoue, M., Rai, S., & Ono, K. J. V. p. Prevalence of Cryptosporidium parvum infection and pattern of oocyst shedding in calves in Japan.2000. *94*(1-2), 27-32.

8. AL-Ezzy, A. I. A., & Kadhim, A. T. Clinical Evaluation for the wet mount preparations versus Ziehl–Neelsen staining modifications for Diagnosis and severity scoring of cryptosporidium parvum in children under 5 years. Paper presented at the Proceedings of 2nd National & 1st International Scientific Conference.2021.
9. Chako, C., Tyler, J., Schultz, L., Chiguma, L., & Beerntsen, B. J. J. o. V. I. MCryptosporidiosis in people: it's not just about the cows.2010. *24*(1), 37-43.
10. Salman, Y. J., Sadek, W. S., & Rasheed, Z. K. J. I. J. C. M. A. S. Prevalence of Cryptosporidium parvum among Iraqi displaced people in Kirkuk city using direct microscopy, flotation technique and ELISA-copro antigen test.2015. *4*(11), 559-572.
11. Chin, J. Control of communicable diseases manual. 2000.
12. Haidar, R. A. Epidemiology of cryptosporidium parvumin in Basra city, Iraq in 2019. Paper presented at the AIP Conference Proceedings.2022
13. Al-Mosa, A. Epidemiological study of the parasitic causes of some intestinal disorders of human in Al-Hilla city. MSc. Thesis Collage of Medicine, Kufa University, 2002.
14. Al-Taie, M. *Epidemiological study of cryptosporidiosis in Diyala province.* M. Sc. Thesis, College of Vet. Med. Baghdad Univ, 1997.
15. Johnston, S. P., Ballard, M. M., Beach, M. J., Causer, L., & Wilkins, P. P. J. J. o. c. m. Evaluation of three commercial assays for detection of Giardia and Cryptosporidium organisms in fecal specimens.2003; *41*(2), 623-626.
16. Nagamani, K., Pavuluri, P., Gyaneshwari, M., Prasanthi, K., Rao, M., & Saxena, N. J. I. J. o. M. M. Molecular characterisation of Cryptosporidium: an emerging parasite.2007; *25*(2), 133-136.

17. Tahira, F., Khan, H., Shukla, I., Shujatullah, F., Malik, M., & Shahid, M. J. J. C. M. H. E. Prevalence of Cryptosporidium in children with diarrhoea in north Indian tertiary care hospital.2012; *2*(3), 136.
18. Oleiwi, M. J. B. U. Prevalence study for main protozoa diarrheal among patients by using microscopically and molecular methods in Babylon province".2015; 23-25.2015.
19. Yacoub, M. M. *Intestinal Protozoa and Cryptosporidium genotypes in North of West Bank/Palestine.* 2014.
20. Mohammad, F. I. J. A.-Q. J. o. P. S. Detecting of virulence factors COWP gene and CP15 gene for Cryptosporidium parvum by polymerase chain reaction (PCR).2018; *23*(2), 39-47.
21. Oskouei, M. M., Fallah, E., Ahmadi, M., Safaiyan, A., Bakhtiyari, S., Naserifar, R., & Dousti, M. J. I. J. o. P. Molecular and parasitological study of Cryptosporidium isolates from cattle in Ilam, west of Iran.2014; 9(3), 435.
22. Al-Robaiee, I., Al-Farwachi, M. J. J. o. A. V., & Research, A. (2014). Direct ELISA aided coprological diagnosis of Cryptosporidium parvum infection in diarrheic neonatal calves in Mosul city, Iraq. *2014*;1(1), 8-10.
23. Kváč, M., Kouba, M., & Vítovec, J. J. V. p. (2006). Age-related and housing-dependence of Cryptosporidium infection of calves from dairy and beef herds in South Bohemia, Czech Republic.2006; 137(3-4), 202-209.
24. Venu, R., Latha, B., Basith, S. A., Raj, G. D., Sreekumar, C., & Raman, M. J. V. P. Molecular prevalence of Cryptosporidium spp. in dairy calves in Southern states of India.2012; *188*(1-2), 19-24.