



Comparison Study about Selected Human Infection of Zoonotic Cryptosporidiosis by Conventional Diagnostic Methods in Karbala Province, Iraq

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Received: 8 April 2021
Accepted: 20 June 2021
Published: 28 June 2021

DOI:
<https://doi.org/10.30539/ijvm.v45i1.1042>



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Cite:
AL-Yasary JTO, Faraj AA. Comparison study about selected human infection of zoonotic cryptosporidiosis by conventional diagnostic methods in Karbala province, Iraq. Iraqi J. Vet. Med. 28 June. 2021; 45(1): 51-55.

A B S T R A C T

This study was carried out to detect the *Cryptosporidium spp* in Karbala province, Iraq from December 2019 to September 2020. Age, sex, and months interference with parasite prevalence were studied. A total of 100 fecal samples were collected from adults and young and from both sexes of human. Fecal samples were subjected to conventional methods (Flotation Methods by Sheather's sugar solution and stained with modified Ziehl-Neelsen) for parasite diagnosis. The result recorded that the infection rate of *Cryptosporidium spp* was 26%. The age group of 2-6 years had the highest infection rate comparing to other age groups ranged from 12-25 years yet was marginal significant ($P < 0.06$). Regarding sex, there was no significant differences in infection rate, although the males recorded numerically higher rate of prevalence. The rate of infection of *Cryptosporidium spp* were varied among months, where in February recorded 46.66% in contrast to 10% recorded in July. It can be concluded that variables studied (age, sex, and months) have no influence on *Cryptosporidium* prevalence in Karbala province.

Keywords: Sheather's sugar solution, modified Ziehl-Neelsen stain, prevalence, *Cryptosporidium*, Karbala province, Iraq

INTRODUCTION

Cryptosporidium, a protozoan parasite belongs to the Phylum Apicomplexa and Family Cryptosporidiidae, is a common cause of diarrhea in man, domestic animals, and wild vertebrates (1). Infection with cryptosporidiosis in man was observed in 1976 (2). The disease belonged to be one of the most serious infection that cause an intestinal infection of man, animals, and birds. *Cryptosporidium* species infected different sites in the body of their host like intestine, stomach, and respiratory system (3, 4). Human and animal may take the infection by eating and drinking polluted water and food with oocysts of this parasite. The food and water increased the incidence and prevalence of

infection especially in less developed and developing countries where human has insufficient of basic infrastructure or fundamental facilities help avoiding food and water polluted with feces (5). Twenty-three species and sixty-one valid genotypes of *Cryptosporidium spp*. have been studied from a wide range including humans, mammals, birds, domestic livestock, wildlife, reptile, amphibians, and fish which can be causing asymptomatic or mild-to severe gastrointestinal disease in its host species (6). This study aimed to investigate human patient infections with cryptosporidiosis by most two conventional methods Sheather's sugar solution and stained with modified Ziehl-Neelsen staining technique.

MATERIALS AND METHODS

Microscopic Examination

The procedures used in this study were reviewed and approved by the Scientific Committee at the University of Baghdad's College of Veterinary Medicine in compliance with animal welfare ethical standards.

A total of one hundred human patient fecal samples were collected from both gender and different ages from Karbala province, during the study period from the beginning of December 2019 to end of September 2020. Each sample was used for flotation method using Sheather's sugar solution (7). Briefly, 5-10 g of a fecal sample were mixed well with 20 mL distilled water in a clean beaker, filtered through four- to six- layer clean gauze after that. The suspension was collected in test tubes and centrifuged at 1500 rpm for 5 min. Discarding of supernatant and making sure not pour off any of the pellets. Filling the tubes with water and spin at 1500 rpm for 5 minutes, then discarded of the supernatant (this step was repeated until the water has appeared clear). The pellet was kept at the bottom of the tubes. Sheather's sugar solution (9 ml) was added to the test tubes and mixed by a wooden stick. Spinning at 1500 rpm for 5 min. One drop was withdrawn from the top surface by pasture pipette and put on the glass slide, then covered with the coverslip and examined under 40× objective lens then 100× oil immersion lens.

Staining with rapid dimethyl sulfoxide-modified acid-fast stain of *Cryptosporidium* oocysts in stool specimens staining technique (8) was as the procedure described by (8). Rectal swabs were collected in Culturettes (Marion Scientific Corp., Kansas City, Mo.). Fecal material was smeared over a 2.5- by 3.0-cm area of a clean, flamed-glass slide and air dried on a warming plate were then done. The slides were prefixed in a Coplin jar of absolute methanol for 5 to 10 sec, then stained in carbolfuchsin-DMSO solution in a Coplin jar for 5 min and rinsed individually in gently running tap water until excess solution no longer ran off each slide (10 to 30 sec per slide). Slides were then placed in the decolorizer-counterstain for 1 min or until a green background appeared and then were rinsed individually under running tap water for 10 sec, drained, blotted, and placed on a warming plate until thoroughly dry (5 or 10 min). A thin film of immersion oil was applied over each smear with an applicator stick. Slides were examined under bright-field low power (10×). The slide examined under light microscope in 100× oil immersion lens for detection of oocysts.

Statistical Analysis

Chi-square (χ^2) test was used for significant comparing between percentage (0.05 and 0.01 probability) in this study. The Statistical Analysis System- SAS (9) program was

used to detect the effect of difference factors in studied factors percentages.

RESULTS AND DISCUSSION

The characteristic morphology of *Cryptosporidium* oocyst was observed by microscopic examination indicated the presence of *Cryptosporidium* spp using Sheather's sugar solution, the oocyst appeared as rounded to oval shape surrounded by thin membrane and contained undistinguished sporozoites and by Rapid Dimethyl Sulfoxide-Modified Acid-Fast Stain the oocyst appeared to be stained purple (Figure 1). This result was compatible with previous research studies (10-12) who observed that the same morphological characteristic of *Cryptosporidium* spp. The measurement of *Cryptosporidium* spp oocyst using ocular micrometer was $4 \mu\text{m} \times 5.2 \mu\text{m}$ which was agreed with (13) who recorded *Cryptosporidium* spp $\mu\text{m} 4 \times 5 \mu\text{m}$ (100×).

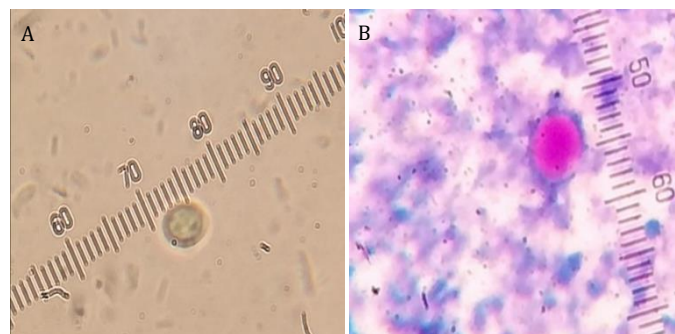


Figure 1. (A) Morphology of *Cryptosporidium* spp. oocyst using Sheather's sugar solution (B) Rapid dimethyl sulfoxide-modified acid-fast stain (100×).

Prevalence of *Cryptosporidium* spp. in man using microscopic examination was showed that among (100) samples were examined (Sheather's sugar flotation and modified Ziehl-Neelsen (mZN) only staining method samples were 26% (26/100), explained that the cryptosporidiosis in human in present study equaled to another previous studies conducted in Iraq which was done (14) in Basra and (15) in Diwaniyah cities as they have been recorded the infection rate 23.8% and 29.29% respectively. Our results also agreed with (16) in Pakistan, in which it recorded 29.88%, but disagreed with another studies in Iraq either higher or lower than present study, The higher rate of infection recorded by (17), in Al-Najaf AL-Ashraf and Baghdad provinces by (18), in which it recorded total percentage of positive result were 58% and 47.33% respectively. (19) mentioned that in Kirkuk the rate of *Cryptosporidium* infection to be lower than the present study which was 16.28%. In some Arab countries prevalence of cryptosporidiosis were 3.4% in Kuwait (20), 17% in Libya (21), 33.9% in Egypt (22) and 8.2% in Sudan (23). In neighboring countries, the prevalence of infection rate was 0.67% in Turkey (24) and was 1.8% in Iran (25). The variation in prevalence of *Cryptosporidium* related to

many factors including variation of the population of the study, age, gender, personal hygiene, drinking or using untreated water, using detected methods, contacting with suspected animal or human and poor economic status of the families may play a key role in the high result of the present study which agreed with (16, 15).

Infection rate of *Cryptosporidium* spp. showed insignificant relation among age groups of patients however, the maximum infection rate showed in age group 2-6 years 44% (11/25), while the minimum rate was among age group 18-25 years 12% (3/25) (Table 1). The result of present study showed an agreement with previous studies in Iraq (14) in Basra in which it was found higher infection rate in children among age group lower than one year (14/50) 28.0% and the lowest infection rate was in age group among one to five years (16/74) 21.6 % but in age group five to fifteen years (2/8) 25.0%. (17) mentioned that in Al-Najaf City a higher rate according to age group among one to ten years (11/50) 22% and lower infection rate among age group fifteen (one to sixteen) (2/50) 4%. In Egypt (22) referred to recording high infection rate in children lower than two years old which was 44.4% and lower prevalence of age group six to twelve years old 27%. The higher infection in children occurs due to their immune system functions which were undeveloped so intake small number of oocysts may result in cryptosporidiosis and repeated low dose infections may stimulate the immunity to *Cryptosporidium* which may protect children tend to have relatively more symptomatic disease than older agree with (26, 27).

Infection rate of *Cryptosporidium* in relation to the sex of infected human was insignificant, in which result showed no significant difference between the rate of infection and the highest rate was in males recorded 27.41% (17/62). The latter percentage from the number infected males, while the lowest percentage 23.68% (9/38) was recorded in the females (Table. 2).

Table 1. Total of infection rate of *Cryptosporidium* spp. among age groups using microscope in man patients

Age (year)	No. examined	No. Positive	% Positive
2-6	25	11	44
6-12	25	7	28
12-18	25	5	20
18-25	25	3	12
Total	100	26	26
χ^2			7.27**

**P≤0.06

Table 2. Prevalence of *Cryptosporidium* spp. according to human sex

Sex	No. examined	No. Positive	% Positive
Males	62	17	27.41
Females	38	9	23.68
Total	100	26	26
χ^2			0.17 NS

**P≤0.67, NS= non-significant

The result of current study agreed with another previous study in Iraq (14) in Basra in which it was found the higher infection rate 24.2% in male and 23.5% in females. The present results disagreed with (28) in Babylon in which it was found that the rate of cryptosporidiosis in males (7.55%) did not vary significantly from females (9.75%). Relationship between gender and infection with *Cryptosporidium* spp. was recorded by (17) in Al-Najaf AL-Ashraf where high prevalence rate in male than female was recorded 16 (55.2%) and 13(44.8%) respectively. The differences in sex in our study explained possibly by that the infection was more in males than females could be due playing of male children in the gardens and farms outdoor area with soil and animals, which can increase the risk of parasite transmission and that agreed with (15, 3).

Prevalence of *Cryptosporidium* spp. in human according to months were insignificant. The results of current study showed that higher infection rate with *Cryptosporidium* 46.66% (7/15) to be in February and the lowest rate of infection was 10% (1/10) while the infection rate in July (Table 3).

Table 3. Total infection rate of *Cryptosporidium* spp. according to months

Months	No. Samples	Positive	%
December 2019	15	4	26.66
January 2020	15	4	26.66
February	15	7	46.66
March	0	0	0
April	0	0	0
May	15	2	13.33
June	10	4	40
July	10	1	10
August	10	2	20
September	10	2	20
Total	100	26	26
χ^2			7.31**

**P≤0.60

The relationship between months variation and infection with cryptosporidiosis was also recorded by (17). In Al-Najaf AL-Ashraf high prevalence rate was found in February 24.1% while the lower rate was in January 13.8%. the above disagreed with (28) in Babylon where it was found that the rate of cryptosporidiosis during September to be 33.75% and lower rate was in January to be 4.54%. Different causes may lead to increase the *Cryptosporidium* infection in the present study including increase of the human exposure to the parasite during autumn and winter due to increasing of picnics, flies and insects spreading and intermittent in the weather lead to change in immunity; in addition to the highest prevalence of *Cryptosporidium* infection was usually associated with the rainy season, and that agreed with (20, 29).

In human the *Cryptosporidium* spp infection rate may be indicated by using microscopic technique but the infection rate of *Cryptosporidium* spp. among age groups, sex, and months of study are not significant.

ACKNOWLEDGEMENTS

My deep gratitude goes to the Head of Department of Parasitology and all members of Department of Parasitology for their cooperation and help.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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تحديد انتشار طفيلي الابواغ الخبيثة في الانسان بالطرائق التقليدية في محافظة كربلاء، العراق

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الخلاصة

هدفت الدراسة الحالية للكشف عن طفيليات الابواغ الخبيثة في الانسان وتحديد المواصفات الشكلية للطفيلي في محافظة كربلاء في العراق بالطرائق التقليدية (تم التطويق بمحلول السكري المشبع وصبغه زيل نلسن المحورة) لدراسة تأثير العمر والجنس والأشهر على معدل الإصابة وكذلك وصف شكل الطفيلي في الانسان. أجريت هذه الدراسة خلال الفترة من بداية شهر كانون الاول (2019) إلى شهر ايلول (2020). تم جمع 100 عينة براز من مختلف الاعمار ومن كلا الجنسين. سجلت نسبة الإصابة بداء الابواغ الخبيثة في الانسان 26%. وان معدل الإصابات بطفيليات الابواغ الخبيثة أظهرت علاقة معنوية بين الفئات العمرية للانسان ، حيث ظهر اعلى معدل الإصابة في الفئات العمرية من 2-6 سنوات 44% ، وكذلك اقل في الفئات العمرية من 6-12 سنة 28% وفي الفئات العمرية من 12-18 سنة كانت نسبة الإصابة 20% بينما كان اقل معدل اصابه بين الفئات العمرية 18-25 سنة 12% وانتشار داء الابواغ الخبيثة بالنسبة لجنس الانسان المصاب ، أظهرت النتيجة عدم وجود فرق معنوي بين معدل الإصابة وكان أعلى معدل عند الذكور حيث سجل 27.41% بينما كانت النسبة منخفضة 23.68% في الإناث. انتشار طفيليات الابواغ الخبيثة في الانسان وفقاً للأشهر الدراسة وأظهرت نتائج الدراسة الحالية اعلى معدل للإصابة بداء الابواغ الخبيثة 46.66% في شباط (2020) وأقل معدل إصابة كان 10% في تموز. الاستنتاج كان معدل الإصابة بطفيليات الابواغ الخبيثة باستخدام الفحص المجهر 26% ، وكان هناك فرق معنوي في معدل الإصابة بطفيليات الابواغ الخبيثة بين الفئات العمرية وأشهر الدراسة ولا يوجد فرق كبير في معدل الإصابة بين الاجناس. نستنتج من هذه الدراسة ان كل من العمر والجنس و الشهور ليس لهم تأثير على معدل انتشار طفيلي الابواغ الخبيثة في محافظة كربلاء.

الكلمات المفتاحية: التطويق بمحلول السكري، صبغة زيل نلسن المحورة ، انتشار، طفيلي الابواغ الخبيثة ، كربلاء العراق