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

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ABSTRACT

Cryptosporidium spp. Parasites were detected in man in Karbala province of Iraq by conventional methods (Flotation Methods by Sheather’s sugar solution and stained with modified Ziehl-Neelsen) to study the effects of age, sex, and months on the infection rate and to record the morphological characterization of Cryptosporidium spp in patients. This study was done through the period from beginning of December 2019 to September 2020. A total of 100 fecal samples were collected from adult and young and from both sexes of human. The result recorded infection rate of Cryptosporidium spp in human in about 26%. Infection rate of Cryptosporidium spp. showed a significant relation among age groups of humans and the maximum infection rate was showed at age group 2-6 years 44% (11/25) and this percentage of infection from the number human samples at this age group, while the minimum rate was among age group 18-25 years 12% (3/25) and this percentage of infection from the number human samples at the age group. Prevalence of cryptosporidiosis in relation to the sex of infected human. The result showed no significant difference between the rate of infection and the highest rate was in males who recorded 27.41% (17/62) and this percentage of infection from the number male samples, while the lowest percentage 23.68% (9/38) was recorded in the females and this percentage of infection from the number female samples. Prevalence of Cryptosporidium spp. in human was according to Months of the year. The results of current study showed that higher infection rate with Cryptosporidium 46.66% (7/15) in February and this percentage of infection from the number of samples at this month. And the lowest rate of infection was 10% (1/10) infection rate in July and this percentage of infection from the number of samples at this month. In Conclusion, human patients indicated that the Cryptosporidium spp infection rate by using microscopic technique to be 26%, significant differences in infection rate of Cryptosporidium spp. among age groups and months of study. There was no significant difference in infection rate between sex.

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
smearred 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 (χ²) 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
ocyst 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 µm × ±5.2 µm which was agreed
with (13) who recorded Cryptosporidium spp µm 4×5 µm
(100×).

![Figure 1](https://via.placeholder.com/150)

**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).

| Age (years) | 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         |

\*\(\chi^2\) \*P≤0.06

| Sex        | No. examined | No. Positive | % Positive |
|------------|--------------|--------------|------------|
| Males      | 62           | 17           | 27.41      |
| Females    | 38           | 9            | 23.68      |
| Total      | 100          | 26           | 26         |

\*\(\chi^2\) \*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 recorded16 (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). 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 rat 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.
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CONFICT OF INTEREST

The authors declare that there is no conflict of interest.

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تحديد انتشار طفيلي الابواغ الخبيئة في الإنسان بالطرق التقليدية في محافظة كربلاء، العراق

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الخلاصة
هدفت الدراسة الحالية للكشف عن طفيليات الابواغ الخبيئة في الإنسان وتحديد المواصفات الشكلية للطفيلي في محافظة كربلاء في العراق بالطرائق التقليدية (تم التطويف بمحلول السكري المشبع وصبغة زيل نيلسون المحورة) للدراسة تأثير العمر والجنس والأيام على معدل الإصابة. أجريت هذه الدراسة خلال الفترة من بداية شهر كانون الأول (2019) إلى شهر أيول (2020). تم جمع 100 عينة براز من مختلف الأعمار ومن كلا الجنسين، سجلت نسبة الإصابة بداء الابواغ الخبيئة في الإنسان 26.7%. ونسبة الإصابة بطفيليات الابواغ الخبيئة أظهرت علاقة معنوية بين الفئات العمرية للإنسان، حيث ظهر أعلى معدل الإصابة في الفئات العمرية من 2-6 سنوات 44.7%، وكذلك أقل في الفئات العمرية من 6-12 سنة 28%، وفي الفئات العمرية من 12-18 سنة كانت نسبة الإصابة 20% بينما كان أقل معدل الإصابة بين الفئات العمرية 18-25 سنة 12ً/1 وانتشار الابواغ الخبيئة بالنسبة للإنسان المصاب، أظهرت النتيجة عدم وجود فرق معنوي بين معدل الإصابة وكان أعلى معدل عند الذكور حيث سجل 46.64%، بينما كانت النسبة منخفضة 23.68% في الإناث. انتشر طفيليات الابواغ الخبيئة في الإنسان وفقًا للأيام، فأظهرت نتائج الدراسة الحالية أعلى معدل الاصابة بداء الابواغ الخبيئة 46.64% في شباط (2020). ونسبة الإصابة كان 10% في تموز. الدراسة أظهرت أن معدل الإصابة بطفيليات الابواغ الخبيئة باستخاذ الفحص المجهر 26%، وكان هناك فرق معنوي في معدل الإصابة بطفيليات الابواغ الخبيئة بين الفئات العمرية والأيام. الدراسة أظهرت أن معدل الاصابة بداء الابواغ الخبيئة في الإنسان كان 26.7%، ونسبة الإصابة بين الجنسين كان 23.68% في الذكور و 46.64% في الإناث.

الكلمات المفتاحية: التطويف بحلول السكري، صبغة زيل نيلسون المحورة، انتشار طفيلي الابواغ الخبيئة، كربلاء، العراق