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

Jihad T O AL-Yasary*, and Azhar A Faraj

Department. of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq

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 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 for 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 ($\chi^2$) 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 $\pm 4 \mu m \times \pm 5.2 \mu m$ which was agreed with (13) who recorded Cryptosporidium spp $\mu m 4 \times 5 \mu m$ (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 crypto sporidiosis 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 infection rate of Cryptosporidium spp. among age groups using microscope in man patients

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>No. examined</th>
<th>No. Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-6</td>
<td>25</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td>6-12</td>
<td>25</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>12-18</td>
<td>25</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>18-25</td>
<td>25</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

\[ \chi^2 \approx 7.27^{*}\]

\[^{*}P<0.06\]

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. examined</th>
<th>No. Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>62</td>
<td>17</td>
<td>27.41</td>
</tr>
<tr>
<td>Females</td>
<td>38</td>
<td>9</td>
<td>23.68</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

\[^{*}P<0.67, \text{NS}= \text{non-significant}\]

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.
ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

15. Al-Diaife RS, Nuha QM, Khawla HS. A study to detect the most important virulence factors of Cryptosporidium parasite samples by PCR. Eurasia J Biosci. 2020; 14: 4649-4652.
تحديد انتشار طفيلي الابواغ الخبيئة في الإنسان بالطرائق التقليدية في محافظة كربلاء، العراق

جهاد طالب عبيد، وأزهار علي فرج
فرع الطفيليات، كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة
هدفت الدراسة الحالية للكشف عن طفيليات الابواغ الخبيئة في الإنسان وتحديد المواصفات الشكلية للطفيلي في محافظة كربلاء، العراق. أجريت هذه الدراسة خلال الفترة من بداية شعبان 2019 إلى شعبان 2020. تم جمع 100 عينة براز من مختلف الأعمار ومن كلا الجنسين وتم التطور بمحلول السكري المشبع وصبغته زيل نلسن المحورة. حيث ظهر أعلى معدل الإصابة في الفئات العمرية من 2-6 سنوات (44٪). ونسبة الإصابة بين الفئات العمرية من 6-12 سنة (28٪) وفي الفئات العمرية من 12-18 سنة كانت نسبة الإصابة (20٪) بينما كانت أقل معدل إصابة بين الفئات العمرية من 18 سنة وفوق. وانتشر تطور الابواغ الخبيئة بالنساء لنساء الإنسان المصابة، حيث أن التطور في العدد ووجود فرق معروفي بين معدل الإصابة وكان أعلى معدل عند الذكور حيث سجل 47.4٪ بينما كانت النسبة منخفضة (23.68٪) في الإناث. انتشر طفيليات الابواغ الخبيئة في الإنسان وفقاً للأشهردراسة وأظهرت نتيجة الدراسة الحالية أعلى معدل الإصابة في شباط 2020 (46.66٪) في شباط 2020، ونسبة الإصابة كان (10٪) في تموز. الانتباه كان معدل الإصابة بطفيليات الابواغ الخبيئة باستخدام الفحص المجهرى (26٪) وكان هناك فرق معروفي في معدل الإصابة بطفيليات الابواغ الخبيئة بين الفئات العمرية ومشكلة القرحة. وبشكل عام، لم يوجد فرق كبير في معدل الإصابة بين الجنسين.

الكلمات المفتاحية: التطويف بمحلول السكري المشبع، سبحة زيل نلسن المحورة، الانتشار، طفيلي الابواغ الخبيئة، كربلاء العراق

AL-YASARY JTO AND FARAJ AA