INCIDENCE AND EXPERIMENTAL INFECTION OF CRYPTOSPORIDIUM BAILEYI IN CHICKEN

M. Th. S. AL-Zubaidi 1, L. I. Kadhim 2, Z. I. Ibrahim 3, A. Sh. Al-Rikabi 4

1Department of Parasitology, Faculty of Veterinary Medicine, University of Baghdad.
2Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, University of Kerbala.
3Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine University of Baghdad.
4Department of Anatomy and Embriology, Faculty of Veterinary Medicine University of Baghdad.

Mohabood24@gmail.com

ABSTRACT

This study was aimed to investigate the incidence of Cryptosporidiosis in 200 fecal samples from slaughtered broiler chicken carcasses in the local markets in some areas of Baghdad city (Al-Hurriya, Al-Kadhimiya and Al-Shuala), during March to May 2017. Three diagnostic techniques used (flotation by Sheather’s sugar solution, staining with Modified Ziehl-Neelsen stain, and measuring of isolated Cryptosporidium oocysts by ocular micrometer) to determine the type of Cryptosporidium species, and for confirm that the isolated species of parasite from infected cases belong to the C. baileyi. Experimental infection done in 18 broiler chicken chicks aged one week divided to three groups first (G1) and second (G2) inoculated orally with, (500, 1000) oocysts per chick respectively, while the third group remain as a control (G3), than pathological lesions detected in some internal organs of infected chicks (trachea, intestine and bursa of Fabricius). The study recorded a total infection rate 35% (75/200) in slaughtered broiler chicken. The result were revealed that the highest rate of infection occurs in April, reached 46% (23/50), while the lowest rate of infection in June, reached 20% (10/50). The experimental study revealed, the infection was occur in all chicks, of (G1) and (G2), and the first clinical signs appear after 7days post infection (PI) which include diarrhea, dullness, anorexia, and increased consumption of water, which represented the incubation period of the parasite, while the shedding of oocysts in feces started after 6-9days PI.

Key Words: Cryptosporidium baileyi. broiler chicken, experimental infection

269
INTRODUCTION
Cryptosporidiosis is a common parasitic disease that affects both humans and animals. It occurs through ingestion of contaminated food and drinking water with mature oocysts. Jackson Clark was the first person who found the parasite in 1895 in the mucous layer of intestine of rat and was called Swarm spores. In 1910, Tyzzer called Cryptosporidium, which it is a Greek term means hidden spores, because the difficulty of diagnosing the four crescent sporozoite in the oocyst, unlike other types of coccidia, they do not contain Sporocyste. (20) The importance of the parasite was increased in 1955 in poultry after the spread of the parasite in turkey fields, which caused losses in a farm in Romania, and recorded high rates of infection with economic losses, then began to pay attention to the classification of this parasite, and its species in the various hosts (19,48). In Iraq, the parasite was first recorded in broiler chickens in 1985 by researchers Al-Attar and Abdul Aziz, (3) in Baghdad city with infection rate 8.8%, and isolate the parasite from the bursa of fabricius, without any clinical signs. Bird species, including poultry, are infected with three species of Cryptosporidium, C.baileyi which their oocysts measuring 6.2 × 4.6 micrometers, it affects the respiratory tract, small intestine, kidneys, bursa of fabricius and cloaca, in poultry, and C. galli, which their oocysts measuring 8.3 × 6.3 micrometers, which affects the real stomach Proventriculus of chickens and birds, and C. meleagridis, which their oocysts measuring 5.2 × 4.6 micrometers, that infects the small intestine of the turkey and can infect humans (1,7,15,20,37,44,47). The parasite can cause up to 25% mortality rate and 100% morbidity rate in broiler chickens, especially in some countries. The parasite can infect chickens, turkeys, pigeons and other wild birds (44). The life cycle of the Cryptosporidium parasite occurs in a single host, which takes approximately 48-72 hours. This cycle is complex and involves two cycles, one of which is asexual and the other is sexual cycle (29,51). The infection occurs after ingestion of contaminated food and water with mature thick-walled oocysts, with the possibility of auto-infection (20). The prepatent period of parasite in poultry range from (3-14) days depending on parasite virulence, number of oocysts, and immunity of the host (14, 25, 44). This study designed to determine the incidence of Cryptosporidium in slaughtered broiler chickens in local markets in some areas of Baghdad province and to conform the isolated species of this parasite from characteristic features of oocyst and from study the pathological lesions in infected organs of experimentally infected chicks.

MATERIALS AND METHODS
Collection of samples: A total of 200 fecal samples collected randomly from intestine of slaughtered broiler chickens in the local markets in some areas of Baghdad province (Al-Hurriya, Kadhimiya and Al-Shula) from the beginning of March until the end of June 2017. The samples were placed in 100 ml clean, sterile sealed containers and sequential numbers were given with the name of the area from which the sample was taken. The specimens were transferred to the parasitology department at the Faculty of Veterinary Medicine / University of Baghdad for laboratory diagnosis.

Examination of samples
Three laboratory methods were used to diagnose oocysts of parasite, Sheather's sugar solution, Modified Zeihl-Neelsen Stain (MZN) and measuring of Cryptosporidium oocysts by ocular micrometer (8, 10, 11, 16, 52).

Measurement of Cryptosporidium oocysts
Ocular Micrometer was used to measure the length and width of parasite oocysts to confirm the type of Cryptosporidium species in the feces of slaughtered chicken carcasses (52) in order to compare them with global measurements of poultry Cryptosporidium species (20).

Isolation and Calculation of the Cryptosporidium oocysts
After isolating and purifying the parasite oocysts which found in the feces of infected slaughtered chicken by using flotation with Sheather's sugar solution according (4,9,16). The purified oocysts from this method, storage in 2.5% potassium dichromate solution v/v, and the number of oocysts calculated in 1 ml of suspended oocysts solution by using haemocytometer slid which used for white blood cells calculation in the eight squares of the two chamber of this slid, then the total
The experimental study was conducted to confirm that the isolated species of parasite from infected cases belong to the \textit{C. baileyi} according to histopathological lesions which occur in the infected organs (trachea, intestine and bursa of Fabricius). The three chicks divided into three groups, each group consisted of six birds. The G1 and G2 were infected orally with one ml of suspended oocysts solution, which containing 500 and 1000 oocysts, respectively, while the G3, act as control group, which inoculated with one ml orally of normal saline solution. The three groups were placed inside cages within a typical poultry breeding hall, which prepared the appropriate heat and ventilation. All chicks groups before experiment examined their feces for detection of \textit{Cryptosporidium} oocysts to ensure that the parasite was not presence. All chicks groups were examined before experiment to ensure that the parasite oocysts were not present.

**Examination of Experiment chicks**

Fecal samples of all three groups chicks were examined after 3 days PI, to confirm the incidence of the infection and the initiation of oocyst shedding, the control groups were monitored for the duration of the experiment and the clinical signs of the infected chicks were observed.

**Histopathological Examination**

Six chickens from all the experiment groups were killed on the 14th day PI. The second group remained after twenty-one days PI. Tissue samples were taken from trachea, small intestine, and bursa of fabricius, and placed in formalin solution 10% for 24 hours for fixation, and histopathological sections were made according (32) for histopathological examination.

**Statistical analysis:**

The Chi-square test was used for the comparison between the results. Differences were considered statistically significant at P<0.05 (49).

**RESULTS AND DISCUSSION**

The results of this study showed that the percentage of total infection of \textit{Cryptosporidium} in fecal samples of slaughtered broiler chicken in the local markets in some areas of Baghdad province was 35% (70/200). There were no significant differences in the percentage of infection in the surveyed areas: Al-Hurriya, Al-Kadhimiya and Al-Shuala, 34.66%, 34% and 36% respectively. (Table 1)

Table 1. Prevalence of \textit{Cryptosporidium} in fecal samples of slaughtered broiler chicken according to the areas

<table>
<thead>
<tr>
<th>Areas</th>
<th>No. of Samples examined</th>
<th>No. of positive</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Hurriya</td>
<td>75</td>
<td>26</td>
<td>34.66</td>
</tr>
<tr>
<td>Al-Kadhimiya</td>
<td>50</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Al-Shuala</td>
<td>75</td>
<td>27</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>70</td>
<td>35</td>
</tr>
</tbody>
</table>

This results agrees with Al-Bayati (6) who found infection rate 21.82% in broiler chickens in Baghdad city, and agrees with Al-Bakri (5) in Nineveh province which found 42.14% of local chickens infected with the parasite, also the result approached with the Kichaw et al. (30) in Morocco, who recorded 24% of chickens infected with \textit{Cryptosporidium}, also the results agrees with the Papadopoulou et al., (36) in Greece, Darabas (18) in Romania, and Shemshadi et al., (46) in Iran which they recorded infection...
rates in broiler chickens reached 24.3%, 22.5% and 23.8% respectively, but the results differed from Al-Attar and Abdul Aziz (3) in Iraq and Kucukerden et al., (31) in Turkey who they recorded infection rates 8.8%, 4.4% respectively in broiler chickens. The variation in incidence of Cryptosporidiosis in broiler chickens in these study may be attributed to many factors, including climate (Temperature and Humidity), conditions of breeding, distribution of fields in the spacing areas (density of breeding fields), type of water sources (treated water or river water which more polluted by Cryptosporidium oocysts). (20). The result of this study showed a significant difference in infection rate according to the months, the highest infection rate 46% (23/50) recorded in April while the lowest rate 20% (10/50) found in June (Table 2). This result agrees with Rahif and Al-Kilani (39) in Baghdad who reported highest presence of Cryptosporidium oocysts in water in spring months and low in the summer months, also the results agrees with Rongjun et al., (43) who recorded the highest infection rate in the spring months 15.6% and observed a significant decline in summer and autumn months reached 2%. While the result disagreed with Goodwin and Brown (23) who found highest infection rates in summer and the lowest rate in winter, due to the exposure of broilers chicken to stress result from high temperature and humidity.

Table 2. prevalence of Cryptosporidium in fecal samples of slaughtered chicken according to the months

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of Examined samples</th>
<th>No. of positive</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>50</td>
<td>20</td>
<td>40 a</td>
</tr>
<tr>
<td>April</td>
<td>50</td>
<td>23</td>
<td>46 a</td>
</tr>
<tr>
<td>May</td>
<td>50</td>
<td>17</td>
<td>34 b</td>
</tr>
<tr>
<td>June</td>
<td>50</td>
<td>10</td>
<td>20 c</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>70</td>
<td>35</td>
</tr>
</tbody>
</table>

Different superscript refers to significant differences at p<0.05

Form and measurement of Cryptosporidium oocyst: By using sheather's sugar solution the oocysts of Cryptosporidium appear transparent circular or oval shapes, surrounded by a bright halo and contain indistinguishable four sporozoites (Fig:2). While the oocysts appeared glowing red by using MZN stain with blue background according to the opposite color used (Fig: 3) this result agreement with Kadir and El-Yassin, (28) and Hunter and Nichol, (26) who found same results.

The results of calibration of isolated Cryptosporidium oocysts, showed that the measurement size of it was 6.1x 4.5 micrometers (Fig: 4) which resemble the global size of C.bailey. This result agrees with Xiao et al, (52) and Fayer and Xiao, (20) who recorded same measurement size of Cryptosporidium species oocysts in poultry.
Experimental Study

The examination of chicks feces in the three groups of experiment starting in the 3th day PI. The first shedding of oocysts reported in 6th and 9th days PI in G1 and G2 respectively, while the first clinical signs, diarrhea, reported after 7th days PI in G2 and in 10th day PI in G1 which represents the duration of incubation period of the parasite, than followed with other clinical signs such as, dullness, anorexia and increased water consumption compared with the control group, this results agrees with several study which found same clinical signs on the infected birds (1,14,20,25,33,38,45).

Gross Lesion: The chicks in experiment divided in two groups for killing, to observe the post mortem changes and histopathological lesion in some internal organs, first group include three chicks from G1, G2, G3 killed in 14th day PI, while second group also include the remained chicks in the three groups (three chicks from each group) killed in 21th days PI. There is no post mortem changes in G3 chicks, while sever changes seen in the G2, compared with G1, which including redness and thickening of intestinal wall with yellow feces also thickening in air sacs with a foam on them, this result agree with the finding of Goodwin, (22 ) and Özkul and Aydin, (35) which recorded same post mortem changes in poultry and birds respectively.

Histopathological lesion: The result of study showed sever histopathological lesion in affected organs, include trachea, small intestine, and bursa of fabricius, in G2 chicks while less pathological changes seen in G1, and without any changes in G3 which represented the control group.

Histopathological lesion in Trachea

The results of microscopic examination of tracheal cross section of G2 chicks after 14 days PI showed deciliation of the mucous epithelium and observation of developmental stages of the Cryptosporidium parasites appear in the form of round or oval structures on the upper surface of the epithelium (fig 5A), also showed same changes in trachea of G1, but less than G2, (fig 5B). Sever pathological changes observe in G1,G2 chicks after 21days PI include sever trachietis which investigated area in the mucous epithelium represented the presence of proliferated of cells of heterozygous which caused severe necrosis accompanied by debris and infiltration of inflammatory cells as well as the presence of mucosal hyperplasia with goblet cell hypertrophy and presence of mucus materials on the surface of the tracheal epithelium, with subcutaneous cell infiltration with plasma cells and heterophils as well as the proliferation of mononuclear cells (fig 6A&B) respectively, while there is no changes in trachea of G3 chicks (fig 7).

Fig 4. Cryptosporidium oocysts calibrated with ocular micrometer x100

Fig (5A&B) Cross section of Trachea of G2 and G1 chicks respectively after 14 days PI, showed deciliation of the mucous epithelium and observe of developmental stages of the Cryptosporidium parasites (Blue arrow) appear round or oval structures on the upper surface of the epithelium with infiltration of inflammatory (Black arrow) and cell residues in G2 H&E stain X20
Fig (6A&B) Cross section of Trachea of G2 and G1 chicks respectively after 21 days PI, showed deciliation of the mucous epithelium and severe necrosis accompanied by debris (Red arrow) in G2 and infiltration of inflammatory cells (Blue arrow) as well as the presence of mucosal hyperplasia with goblet cell hypertrophy (Yellow arrow) and presence of mucinus materials (Black arrow) on the surface of the tracheal epithelium H&E stain X20.

Fig 7. Cross section of the trachea of control group shows the normal appearance of the epithelial layer, H&E stain, x 20

This result agreed with; (17,23,24,33,34) who observed the developmental stages of *C. baileyi* on the upper surface of epithelial layer of trachea of broiler chickens and showed that the characteristic histopathological changes were the loss of cilia and the destruction of epithelial layer, with congestion of capillaries and the presence of mucous in the mucous epithelium sometimes.

**Histopathological lesion in Small Intestine**

The results of histopathological lesions of the experimental infection on small intestine showed severe epithelial distraction accompanied with widespread necrosis led to loss of mucous membranes with the accumulation of debris cell necrosis with hyperplasia of goblet cells and presence of developmental stages of the parasite, as well as infiltration of sub mucosa layer with mononuclear cells (macrophages and plasma cells) on the epithelial surface in chicks of G1 and G2 after 14days and 21days PI (fig 8,9A & B) respectively, while there is no changes in trachea of G3 chicks (fig 10).

Fig (8A&B) Cross section of small intestine of G2 X20 and G1X40chicks respectively after 21 days PI, epithelial distraction accompanied with widespread necrosis led to loss of mucous membranes (Blue arrow) with a number of developmental stages of the parasite(Black arrow). H&E stain.
Fig (9A&B) Cross section of small intestine of G2 X20 and G1 X40 chicks respectively after 21 days PI, severe epithelial distraction accompanied with widespread necrosis led to loss of mucous membranes with the accumulation of debris cell necrosis (Blue arrow) with a number of developmental stages of the parasite (Black arrow), as well as infiltration of sub mucosa layer. H&E stain

Fig 10. Histopathological Cross section of the small intestine of control group shows the normal appearance of the epithelial layer, H&E stain, x 40

These results agreed with several studies which recorded same pathological lesions in small intestine of infected poultry and birds with cryptosporidiosis, and confirmed the occurrence of similar lesions attributed to C. baileyi infection on the peaks of intestinal villi, and that the infiltration of inflammatory cells in the layers of the intestine is only a response caused by the extensive damage and destruction of the epithelial cells. (1,12,13,20,21,22,27,35,40,44,50).

Histopathological lesion in Bursa of Fabricius

The study showed some pathological lesion in bursa of fabricius of experimentally infected chicks in G1 and G2 after 14 and 21 days PI, include hypertrophy and hyperplasia of bursal epithelial cell, and found some developmental stages of the parasite on the upper surface of the bursa, as well as infiltration of mononuclear cells (plasma cells) (fig 11, 12A &B) while there is no pathological changes in bursa of fabricius of G3 chicks (fig 13). These pathological lesion agrees with Goodwin and Blaghum et al., (13), Rhee et al., (41) in chicken and Alex and Marcelo, (1) in birds which recorded same lesion in bursa of fabricius of infected chicken and birds with cryptosporidiosis.

Fig (11A&B) Cross section of bursa of fabricius of G2 and G1 chicks respectively after 14 days PI, hypertrophy and hyperplasia of bursal epithelial cell (Blue arrow), and found some developmental stages of the parasite (Black arrow) on the upper surface of the bursa, as well as infiltration of inflammatory cells (Red arrow) H&E stain X40
Acknowledgment
The authors are very grateful to Professor Dr. Mohamed Jawed and Assistant Professor Dr. May Hamed Kwan for the technical support provided.

REFERENCES
5- Al-Bakri, H. S. 2012. Detection of Cryptosporidium baileyi oocysts in the feces of domestic chicken in Nineveh province. Iraqi Journal of Veterinary Sciences (Proceedings of the Sixth Scientific Conference, Faculty of Veterinary Medicine, Mosul University), 26 (2): 159-163

277