INCIDENCE AND EXPERIMENTAL INFECTION OF CRYPTOSPORIDIUM BAILEYI IN CHICKEN

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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 Zeihl-Neelsen stain, and measuring of isolated *Cryptosporidium* oocysts by ocular micrometer) to determine the type of *Cryptosporidium* species, and for conform 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**

مجلة العلوم الزراعية العراقية -2018 :49(2):269-278 الزبيدي واخرون

حدوث الاصابة والاصابة التجريبية بطفيلي الكربتوسبوريديوم بيلي في الدجاج

محمد ثابت صالح الزبيدي لطيف ابراهيم كاظم زينب اسماعيل ابراهيم احمد شمخي الركابي
استاذ مساعد استاذ مساعد استاذ مساعد مدرس مساعد

المستخلص

هدفت الدراسة الحالية الى التحري عن حدوث مرض الابواغ الخبيئة Cryptosporidiosis في (200) عينة براز من الدجاج المذبوح في الأسواق المحلية لبعض مناطق مدينة بغداد (الحرية، الشعلة والكاظمية)،للفترة من بداية شهر اذار إلى نهاية شهر أيار2017، من خلال استخدام ثلاث تقنيات تشخيصية (التطويف بالمحلول الشيذر السكري، الصبغ بصبغة الزيل نلسن المحورة و قياس اكياس بيض الطفيلي المعزولة بالمقياس العيني الدقيق، لتحديد نوع طفيلي البوغ الخبيء *Cryptosporidium* ، وللتاكد من نوع الطفيلي المعزول من الحالات المصابة، احدثت اصابة تجريبية في 18 فرخ دجاج لحم بعمر أسبوع واحد والتي قسمت الى ثلاث مجاميع متساوية الاولى والثانية جرعت (1000،500) كيس بيضة/ فرخ على التوالي، فيما اعتبرت المجموعة الثالثة مجموعة سيطرة، سجلت التأثيرات المرضية التي حدثت في الأعضاء الداخلية (الأمعاء، القصبة الهوائية و جراب فبر يشيا) للافراخ المصابة، استخدم التوصيف الشكلي والقياس لأكياس بيض الطفيلي المعزولة بالإضافة الى الآفات المرضية التي وصفت في المقاطع النسيجية للاصابة التجريبية، والتي من خلالها تم تشخيص النوع *C. baileyi* ، سجلت الدراسة نسبة إصابة كلية بلغت 35% (200/75) في دجاج اللحم المذبوح، وبلغت اعلى نسبة للاصابة في شهر نيسان 46% (50/23) واقلها في شهر حزيران 20%(50/10). اظهرت الدراسة التجريبية حدوث الاصابة في جميع افراخ المجموعة الاولى والثانية، واول علامات سريرية ظهرت في اليوم السابع بعد الاصابة والتي شملت الاسهال، الخمول، فقدان الشهية و الزيادة في استهلاك الماء، والتي تمثل فترة حضانة الطفيلي، بينما كانت بداية عملية طرح اكياس البيض في البراز 6–9 ايام بعد اعطاء الاصابة.

الكلمات المفتاحية : البوغ الخبيء بيلي، دجاج اللحم، الاصابة التجريبية

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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 x 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

number of oocysts per 1ml calculate according to the following equation: (2) (Fig: 1)

Number of oocysts in 1 ml = (1000 x calculated oocysts number) / 8

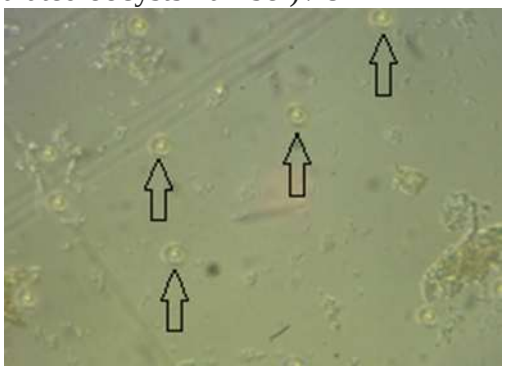


Fig 1. *Cryptosporidium* oocysts calculated by haemocytometer x40

Experimental Study

The experimental study was conducted to conform that the isolated species of parasite from infected cases belong to the *C.baileyi* according to histopathological lesions which occur in the infected organs (trachea, intestine and bursa of Fabricius). (20) For the experimental infection (18) chicks of broiler chickens (Rose type) aged one week and their weights 100-110 gm were used. These 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 *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 fromr 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 *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)

Table1. Prevalence of *Cryptosporidium* in fecal samples of slaughtered broiler chicken according to the areas

Areas	No. of Samples examined	No. of positive	Percentage%
Al-Hurriya	**75**	**26**	**34.66**
Al-Kadhmiya	**50**	**17**	**34**
Al-Shuala	**75**	**27**	**36**
Total	**200**	**70**	**35**

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 *Cryptosporidium*, also the results agrees with the Papadopoulou *et al.*, (36) in Greece, Darabus (18) in Romania, and Shemshadi *et al*., (46) in Iran which they recorded infection

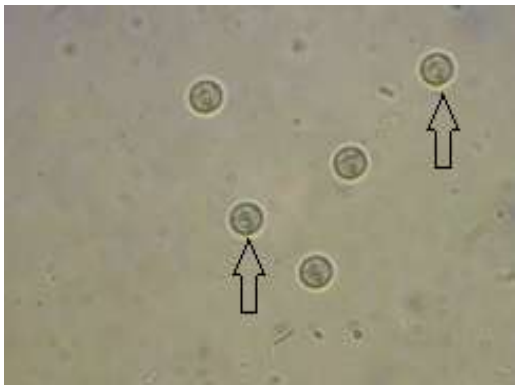
rates in broiler chickens reached 24.3%, 22.5% and 23.8% respectively, but the results differed from Al-Attar and Abdul Aziz (3) in Iraq and Kucukerden *et al*., (31) in Turkey who they recorded infection rates 8.8%, 4.4% respectively in broiler chickens. The variation in incidence of Cryptosporidiosis 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.

Table2. prevalence of *Cryptosporidium* in fecal samples of slaughtered chicken according to the months

Months	No. of Examined samples	No. of positive	Percentage%
March	**50**	**20**	**40** [a]
April	**50**	**23**	**46** [a]
May	**50**	**17**	**34** [b]
June	**50**	**10**	**20** [c]
Total	**200**	**70**	**35**

Different superscript refers to significant differnces 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 undistinguishable 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.



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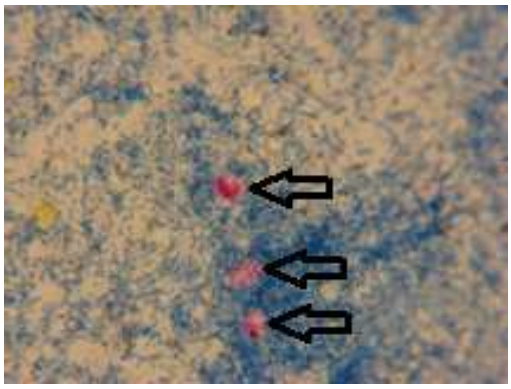

(3)

Fig 2. *Cryptosporidim* oocysts isolated by sheather's sugar solution x100

Fig 3. *Cryptosporidim* oocysts in fecal smear stained with MZN x40

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.

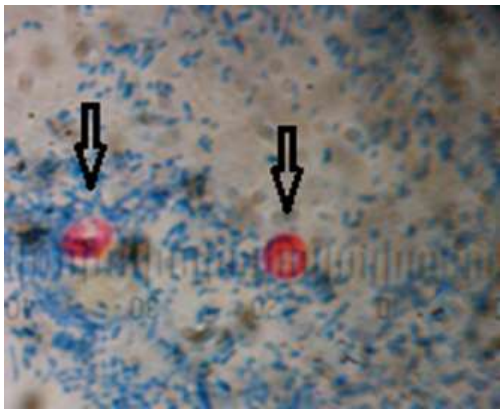

Fig 4. *Cryptosporidim* oocysts calibrated with ocular micrometer x100

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 *Cryptosoporidium* 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 mucinus 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).

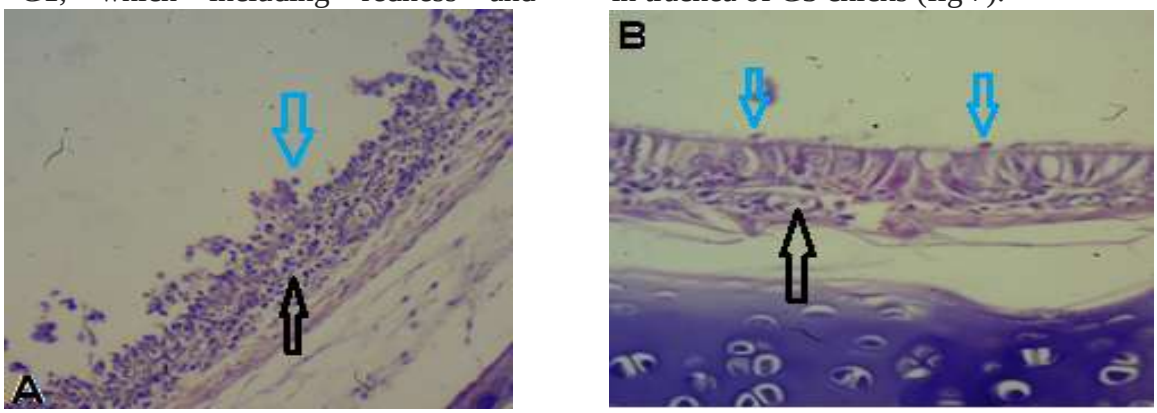

Fig (5A&B) Cross section of Trachea of G2 and G1 chicks respectively after 14 days PI, showed deciliation of the mucous epithelium and observe of developmental stages of the *Cryptosoporidium* parasites (Blue arrow) appear round or oval structures on the upper surface of the epithelium with infiltration of inflammatory (Black arrow) and cell residues in G2 H&E stain X20

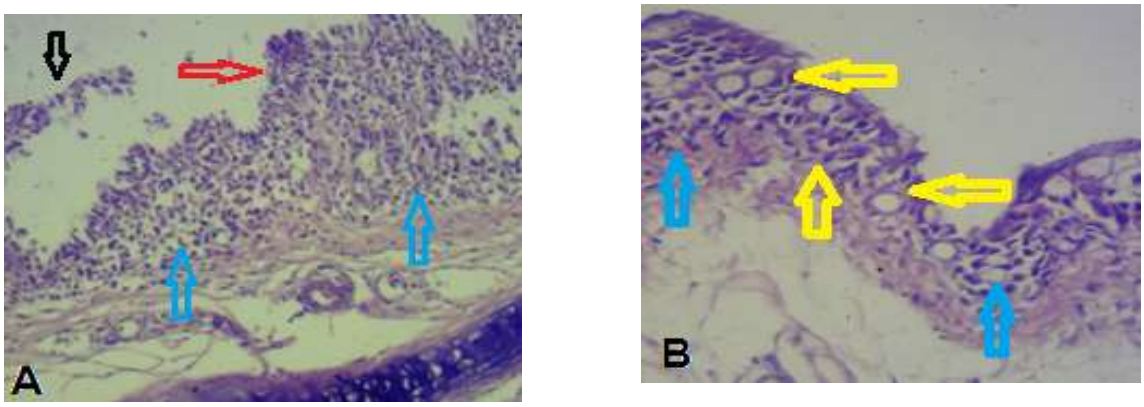

Fig (6A&B)Cross section of Trachea of G2 and G1 chicks respectively after 21 days PI, showed deciliation of the mucous epithelium and severe necrosis accompanied by debris (Red arrow) in G2 and infiltration of inflammatory cells (Blue arrow) as well as the presence of mucosal hyperplasia with goblet cell hypertrophy (Yellow raw) and presence of mucinus materials (Black arrow) on the surface of the tracheal epithelium H&E stain X20

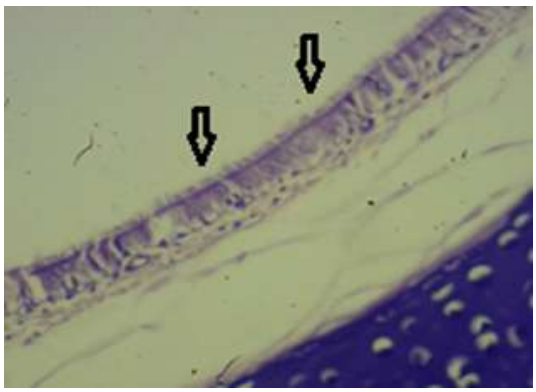

Fig 7. Cross section of the trachea of control group shows the normal appearance of the epithelial layer, H&E stain, x 20

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).

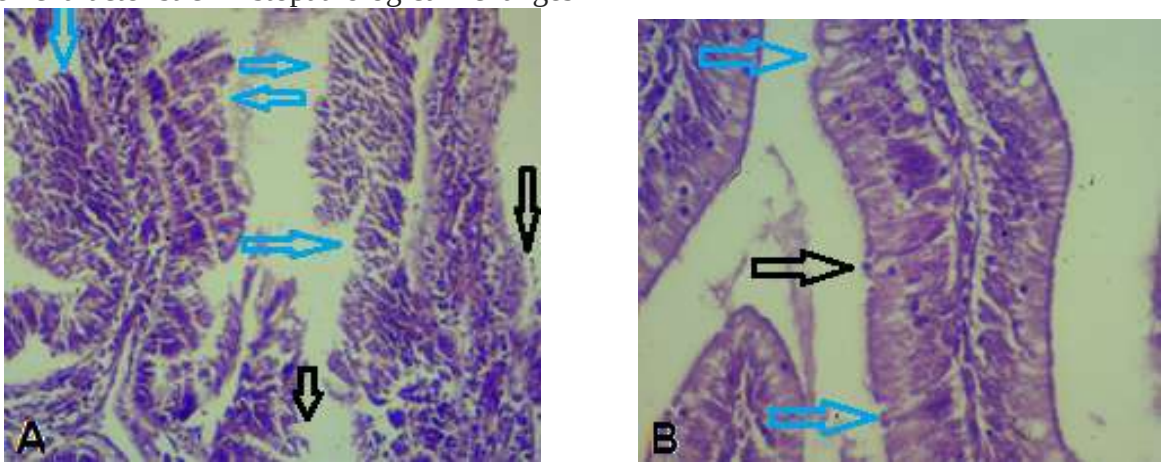

Fig (8A&B) Cross section of small intestine of G2 X20 and G1X40chicks respectively after 21 days PI, epithelial distraction accompanied with widespread necrosis led to loss of mucous membranes (Blue arrow) with a number of developmental stages of the parasite(Black arrow). H&E stain

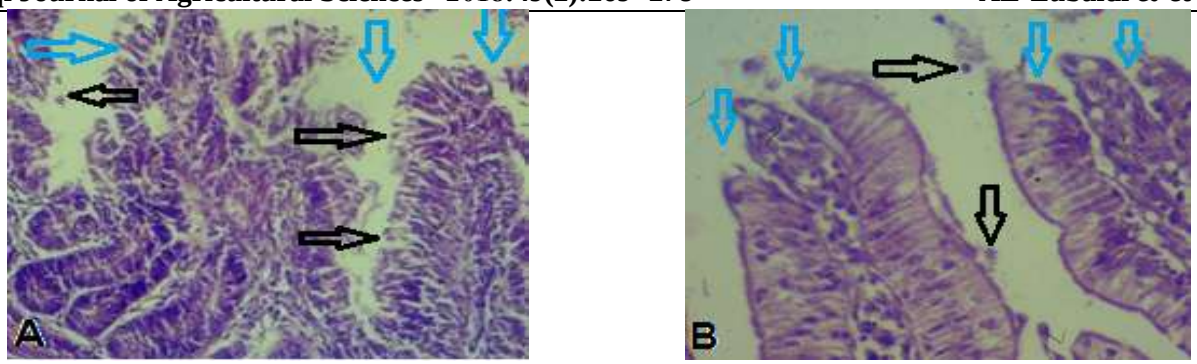

Fig (9A&B) Cross section of small intestine of G2 X20 and G1X40chicks respectively after 21 days PI, severe epithelial distraction accompanied with widespread necrosis led to loss of mucous membranes with the accumulation of debris cell necrosis(Blue arrow) with a number of developmental stages of the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

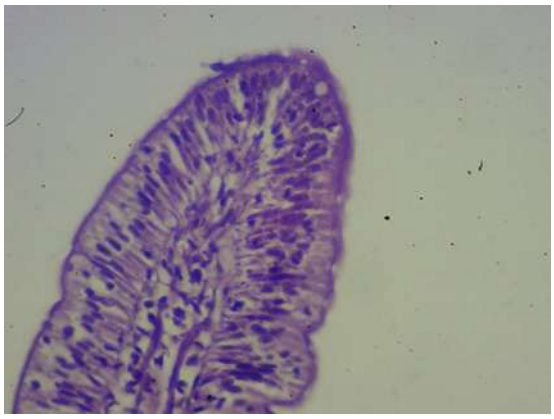

Fig 10. Histopathological Cross section of the small intestine of control group shows the normal appearance of the epithelial layer, H&E stain, x 40

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.

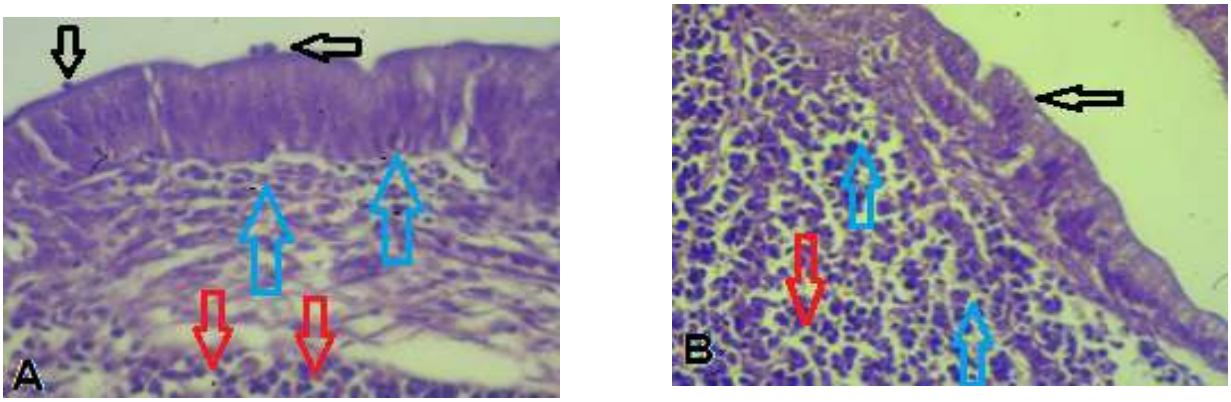

Fig (11A&B) Cross section of bursa of fabricius of G2 and G1chicks respectively after 14 days PI, hypertrophy and hyperplasia of bursal epithelial cell (Blue arrow), and found some developmental stages of the parasite (Black arrow)on the upper surface of the bursa, as well as infiltration of inflammatory cells (Red arrow) H&E stain X40

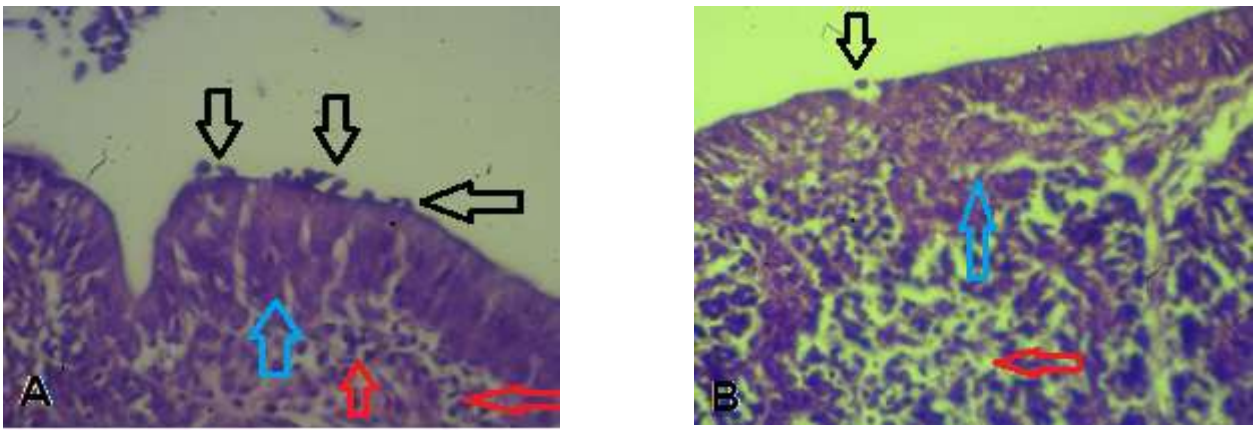

Fig (12A&B) Cross section of bursa of fabricius of G2 and G1chicks respectively after 14 days PI, sever hypertrophy and hyperplasia of bursal epithelial cell (Blue raw), and found some developmental stages of the parasite (Black raw)on the upper surface of the bursa, as well as sever infiltration of inflammatory cells (Red raw) H&E stain X40

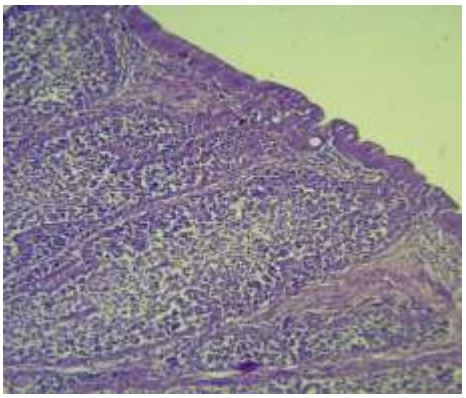

Fig 13. Histopathological Cross section of the bursa of fabricius of control group shows the normal appearance, H&E stain, x 20

The current study proved that the isolated species of *Cryptosporidium* from slaughtered broiler chicken according global and local measurements of oocysts and the histopathological lesion in infected organs (trachea, small intestine, and bursa of fabricius) of experimental infected chicks belong to the *Cryptosporidium baileyi* which isolated from the field study.

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