Basrah Journal of Veterinary Research,Vol.17, No.3,2018
Proceeding of 6th International Scientific Conference,College of Veterinary Medicine
University of Basrah,Iraq

ISOLATION OF *Cryptosporidium* OOCYSTS FROM SLAUGHTERED BROILER CHICKEN AND EXPERIMENTAL INFECTION IN CHICKS

Atheer Kareem Kadhim , Mohammed Thabit Salih Al-Zubaidi

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

Key Words: *Cryptosporidium,* Oocyst,, Chicks.

Corresponding Author: mohabood24@gmail.com

ABSTRACT

The aim of current study was investigate the isolation of *Cryptosporidium* oocysts from 93 fecal samples of infected slaughtered broiler chicken in local markets of some areas in Baghdad city, during the period from the beginning of November 2017 to end of April 2018. In this study three diagnostic techniques used, flotation by Sheather's sugar solution, isolation and measuring of *Cryptosporidium* oocysts (by ocular micrometer). For conform that the isolated species of the parasite from infected cases belong to the *Cryptosporidium baileyi*, experimental infection done in, broiler chicken chicks aged one week to study the histopathological lesion in infected organs (intestinal, trachea, and bursa of Fabricius). The results of calibration of isolated oocysts, showed that the mean of measurement size of *Cryptosporidium* oocysts was 6.2x 4.7 micrometers.

INTRODUCTION

Cryptosporidiosis is considered as a zoonotic parasitic disease caused acute enteritis to the different types of animals and humans, the parasite detected by Jackson Clark in 1895 in intestinal mucosa of rat (1). Tyzzer in 1910, called *Cryptosporidium* on this parasite which it is a Greek term means hidden spores, because the difficulty of diagnosing the four crescent sporozoite in the oocyst, which differ from other types of coccidia, they do not contain Sporocyste (2). The infection occurs through ingestion of mature oocysts with contaminated food and drinking water. The importance of this parasite was increased in 1955 in poultry after the spread of it in turkey fields, and the parasite caused high morbidity and mortality rates in birds in

Romania, with economic losses, then began give attention to the classification of *Cryptosporidium*, and its species in the various vertebrates' hosts (3, 4, 5).

The parasite recorded for the first time in Iraq, in broiler chickens in Baghdad city by Al-Attar and Abdul Aziz (6) which seen the parasite with infection rate 8.8%, and isolate the *Cryptosporidium* from the bursa of fabricius, without any clinical signs. The species of parasite which infect birds including poultry, are three species, *Cryptosporidium galli*, which their oocysts measuring 8.3 × 6.3 micrometers, which affects the real stomach Proventriculus of chickens and birds, *C. meleagridis*, which their oocysts measuring 5.2 × 4.6 micrometers, that infects the small intestine of the turkey and can infect humans, and *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 (2, 7, 8, 9).

The current study was designed to determine the causative species of cryptosporidiosis in slaughtered broiler chickens from calibration of oocysts and study the histopathological changes in site of infection (small intestine, trachea, and bursa of fabricius) in experimentally infected broiler chicken chicks.

MATERIAL AND METHODS

Samples collection

Fecal samples collected from infected slaughtered broiler chicken in local markets of some areas in Baghdad city, during the period from the November 2017 to end of April 2018. The fecal samples were tacked directly from intestine content in a clean plastic container (50ml size) and given sequential numbers. All information for the animal included date of sampling, case history and clinical signs (if found) and the name of region were recorded on containers of the samples, and these samples were transported in refrigerated bag to the laboratory of parasitology/College of Veterinary Medicine-University of Baghdad.

Laboratory examination

1. Floatation using Sheather's sugar solution

Fecal samples (2 g) were mixed will with 20 ml distilled water in a clean flask. The mixture was then filtered through four layers of clean gauze to remove the fecal debris. Afterwards, the suspension was collected in test tubes and was centrifuged (2700 round/ minute) for 15 minutes. Then the supernatant was discarded, and few amount of the suspension was kept with the sediment. Sheather's sugar solution (9 ml) was added to test tubes and mixed well then centrifuged with the same rounds and, then the surface layer was separated which contain the oocysts. One drop of the surface layer was withdrawn by Pasture pipette and run on the a clean glass slide then covered with the cover slip and scanned under light microscope ×40 and ×100 (10).

2-Ocular micrometer was used to determine the oocysts calibration or measurements according to (11).

3-Isolation and counting of oocysts for experimental infection:

Fecal samples (5 g) was mixed with 20 ml distilled water in a clean flask then was filtered through four layers of clean gauze to remove the fecal debris. After that, the suspension was collected in test tubes and were centrifuged (2700 round/ minute) for 15 minutes.

a-The supernatant was discarded, and few amount of the suspension was kept with the sediment and mixed well with Sheather's sugar solution (9 ml) then centrifuged with the same procedure mentioned previously.

b-All the supernatant was collected then diluted with distilled water at a rate of 1:10 to prevent the effect of Sheather's sugar solution on the oocysts.

c-The diluted solution was sedimented by centrifugation (2700 round/ minute) for 15 minutes.

d-The sediment was collected in a clean test tube and added an equal volume of 1% sodium Hypochlorate Solution, mixed well and left for 5 minutes and then added a double volume of distilled water gradually on the wall of the test tube by a syringe of

1 ml size. A clear layer was separated above the solution, pulled by a Pasteur pipette and moved to other test tubes.

e-The clear layer washed by centrifugation several times until the disappearance of the chlorine smell from the solution.

f-The oocysts which isolated by this method from infected cases storage in 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: Number of oocysts in 1 ml = (1000 x calculated oocysts number) / 8. (12, 13, 14) (Fig: 1).

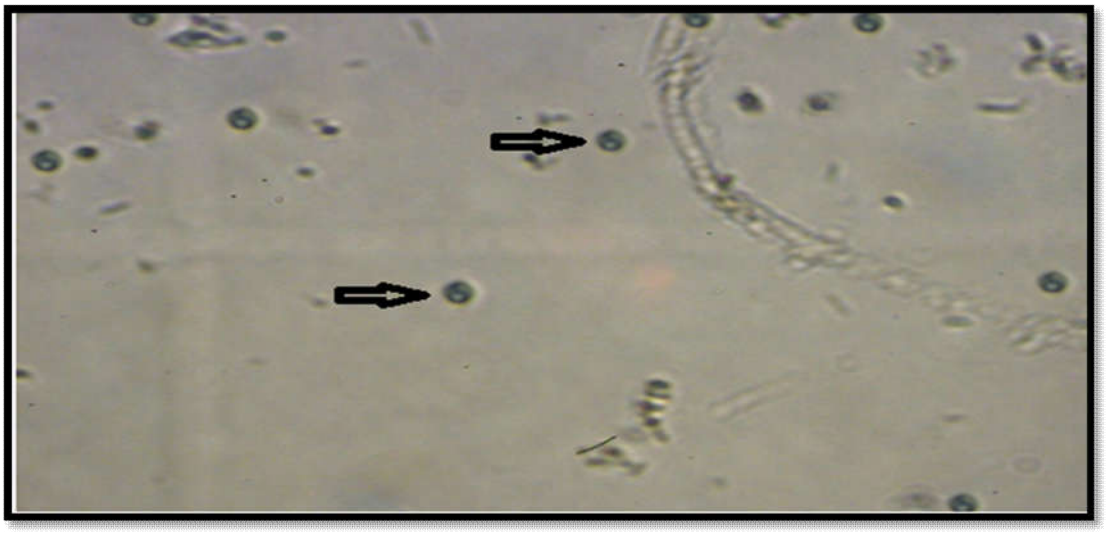


Fig: (1). *Cryptosporidium* oocysts calculated by haemocytometer x40

4-Experimental Study

The experimental infection was conducted to study the histopathological effects of parasite in small intestine, trachea and bursa of fabricius of experimentally infected broiler chicken chicks, and observe the developmental stages of the parasite in these infected organs and record the pathogenic lesions to identification of the parasite type, by presence of these stages of it in the specific site of infection.

For the experimental infection (12) chicks of broiler chickens (Rose type) aged one week and their weights 100-120 g were used. These chicks divided into two groups, each group consisted of six birds. The first group G1 infected orally with one ml of suspended oocysts solution, which containing 1000 oocysts (15, 16), while the second group G2, act as control group, which inoculated orally with one ml of normal saline solution. The two 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.

Examination of Experiment infected chicks

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

Histopathological Examination of infected chicks

Three chickens from each groups of experiment were killed on the 9th day PI. The second group remained after 14 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 (17) for histopathological examination.

RESULTS

The result of study recorded shape and calibration of *Cryptosporidium* oocyst, through using sheather's sugar solution the *Cryptosporidium* oocysts appear transparent oval shapes, surrounded by a bright halo and contain undistinguishable four sporozoites (Fig: 2). The results of calibration of isolated oocysts, showed that the mean of measurement size of parasite oocysts was 6.2x 4.7 micrometers (Fig: 3)

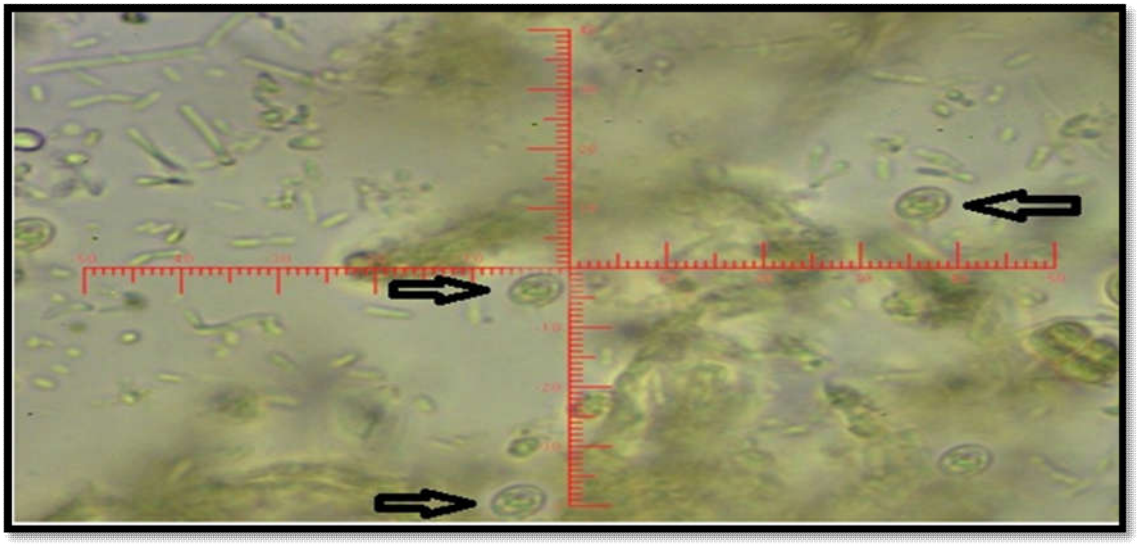


Fig: 2. *Cryptosporidim* oocysts isolated by sheather's sugar solution x100

Fig: 3. *Cryptosporidim* oocysts calibrated with ocular micrometer x100

The Results of Experimental Study:

The two groups of chicks of experiment, starting examined their feces in the 3th day PI. The shedding of oocysts in infected group G1, started in 4th days PI, while the first clinical signs, diarrhea, appear in the 6th days PI, than followed with other clinical signs such as, dullness, increased water consumption, anorexia, while there is not found any clinical signs on the control group G2.

Gross Lesion

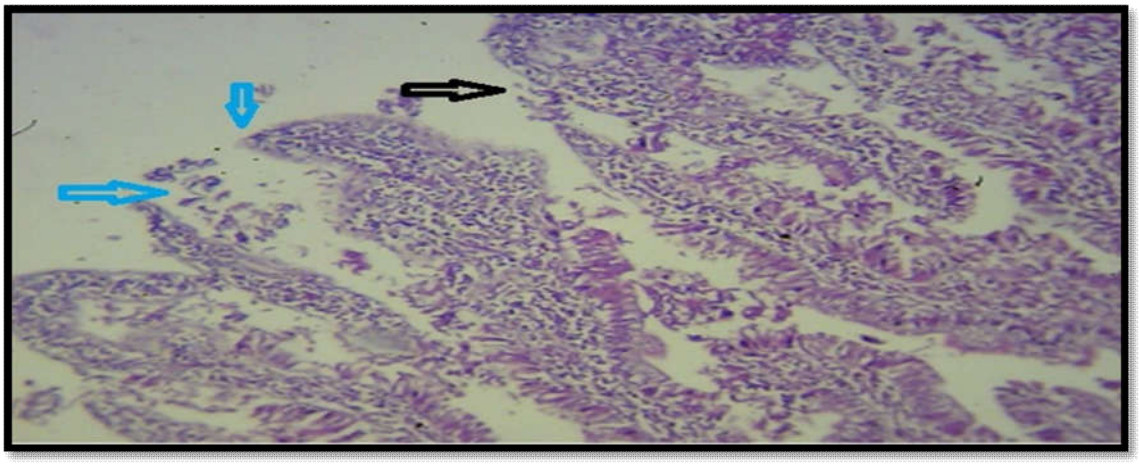
The experiment infected and not infected or control group chicks divided in two groups for killing, to seen the post mortem changes and histopathological lesion in some internal organs, (intestine, trachea and bursa of fabricius), first killed group include three chicks from G1and G2 in the 9th day PI, while second group also include the remained 6 chicks in the two groups killed in 14th days PI. There is sever changes seen in the G1, while no post mortem changes in G2 chicks, these changes including redness and thickening of intestinal wall with yellow feces also thickening in air sacs.

Histopathological lesion in organs of experimentally infected chicks:

The result of study showed sever histopathological lesion in affected organs, include small intestine, trachea and bursa of fabricius, in G1chicks, while without any histopathological changes in G2 chicks, which represented the control group.

Small Intestine Lesions

The intestinal histopathological lesions of the experimentally infected chickse showed severe epithelial distraction accompanied with widespread of necrosis leding 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 on the upper surface, as well as infiltration of sub mucosa layer with mononuclear cells (macrophages and plasma cells) in chicks of G1, after 9days and 14days PI (fig 4, 5) respectively, while there is no changes in intestine of G2 chicks (fig 6).



Fig; 4. Cross section of small intestine of G1 chicks after 9 days PI, widespread of necrosis with epithelial distraction accompanied which led to loss of mucous membranes(Blue arrow), with a number of developmental stages of the parasite (Black arrow), H&E stain X20.

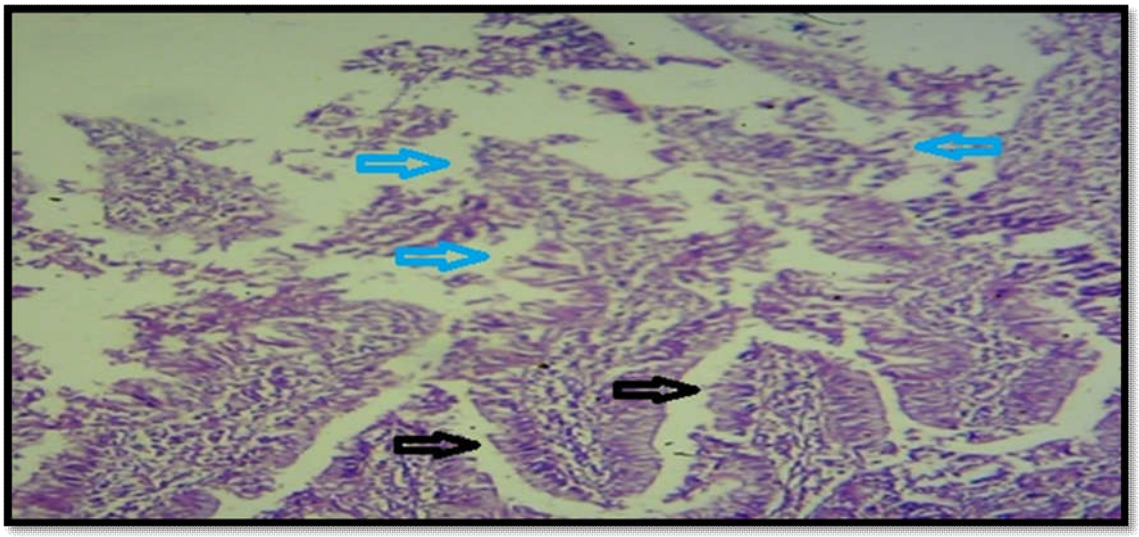


Fig: 5. Cross section of small intestine of G1 after 14 days PI, severe epithelial distraction of mucous membranes with the accumulation of debris cell necrosis with widespread necrosis areas (Blue arrow), with a several developmental stages of the parasite (Black arrow), H&E stain X20.

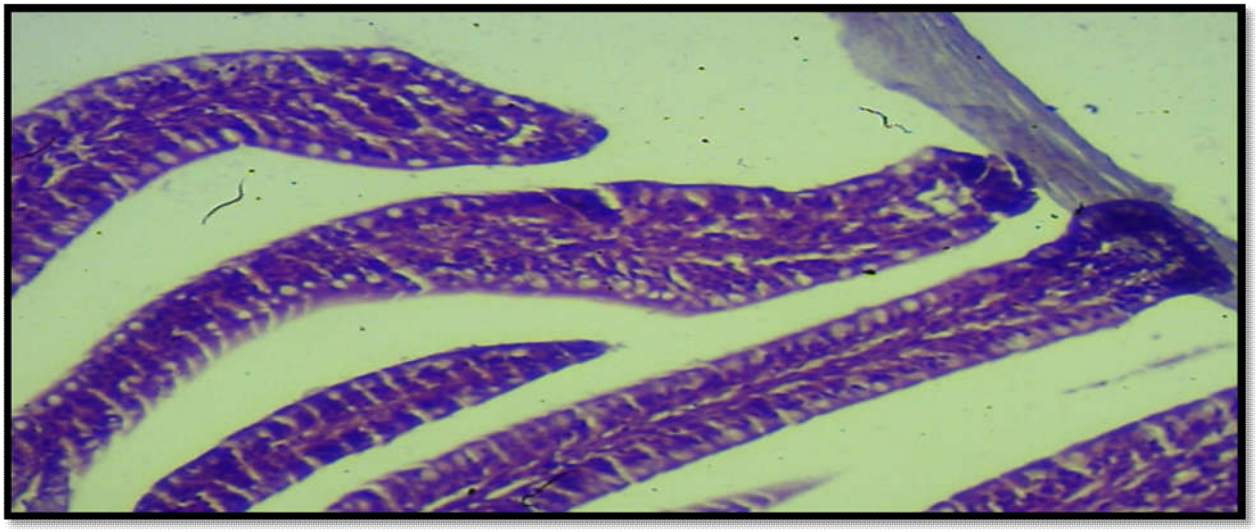


Fig: 6. Cross section of the small intestine of control group G1 shows the normal appearance of the epithelial layer, H&E stain, x 20

Tracheal Lesions

The lesion of tracheal cross section of G1 chicks after 9 days PI recorded several developmental stages of the parasites appear oval or round structures on the upper surface of the epithelium, with deciliation of the mucous epithelium (fig: 7), while sever pathological changes observe in chicks after 14days PI include sever trachietis and proliferated of epithelial layer, with mucinus materials on the surface of the tracheal epithelium and 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, with subcutaneous cell infiltration with plasma cells and heterophils as well as the proliferation of mononuclear cells (fig: 8), while there is no any pathological lesions in trachea of G2 chicks (fig: 9).

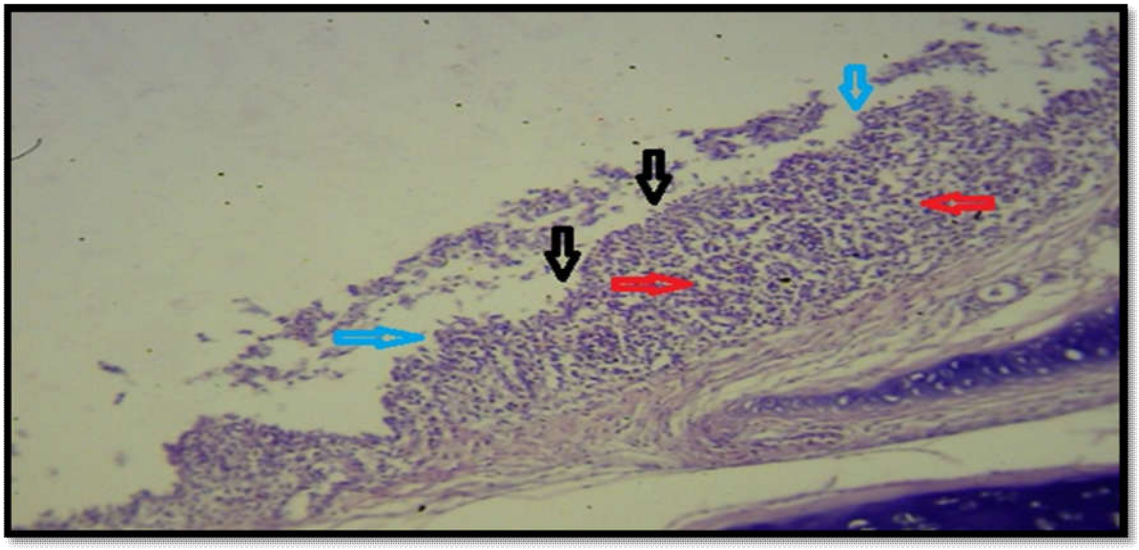


Fig: 7. Cross section of Trachea of G1 after 9 days PI, showes of round or oval structures of developmental stages of the parasites (Black arrow),, with deciliation of the mucous epithelium appear on the upper surface of the epithelium(Blue arrow), with infiltration of inflammatory cell (Red arrow) H&E stain X20.

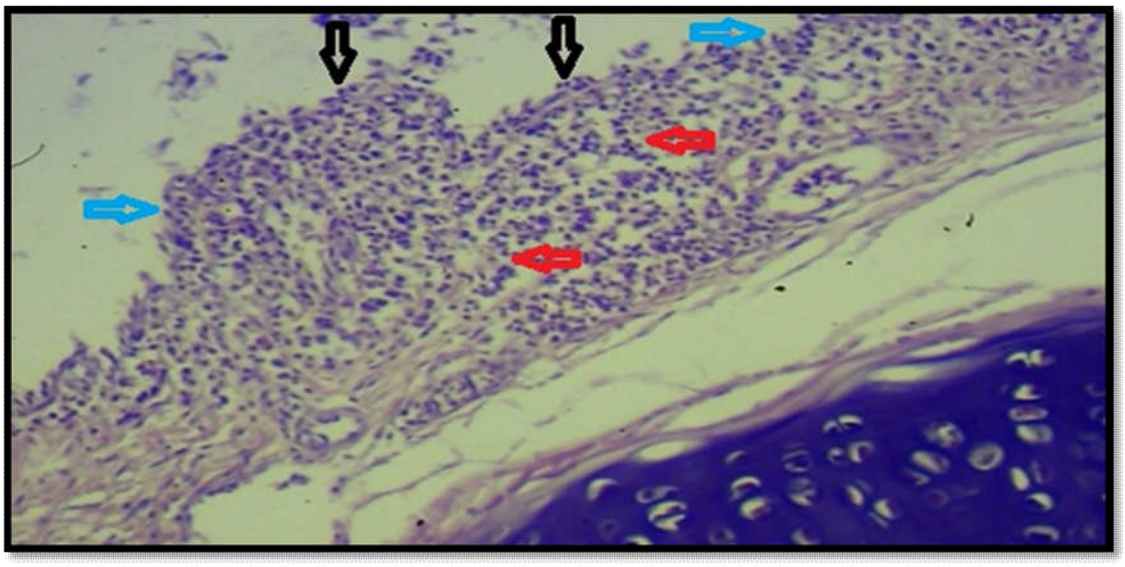


Fig: 8. Cross section of Trachea of G1 chicks after 14 days PI, showed the parasite stages on the upper surface with severe necrosis accompanied by debris (Black arrow), and wide deciliation of the mucous epithelium (Blue arrow), and infiltration of inflammatory cells (Red arrow) as well as the presence of mucosal hyperplasia with goblet cell hypertrophy, with presence of mucinus materials on the surface of the tracheal epithelium H&E stain X20

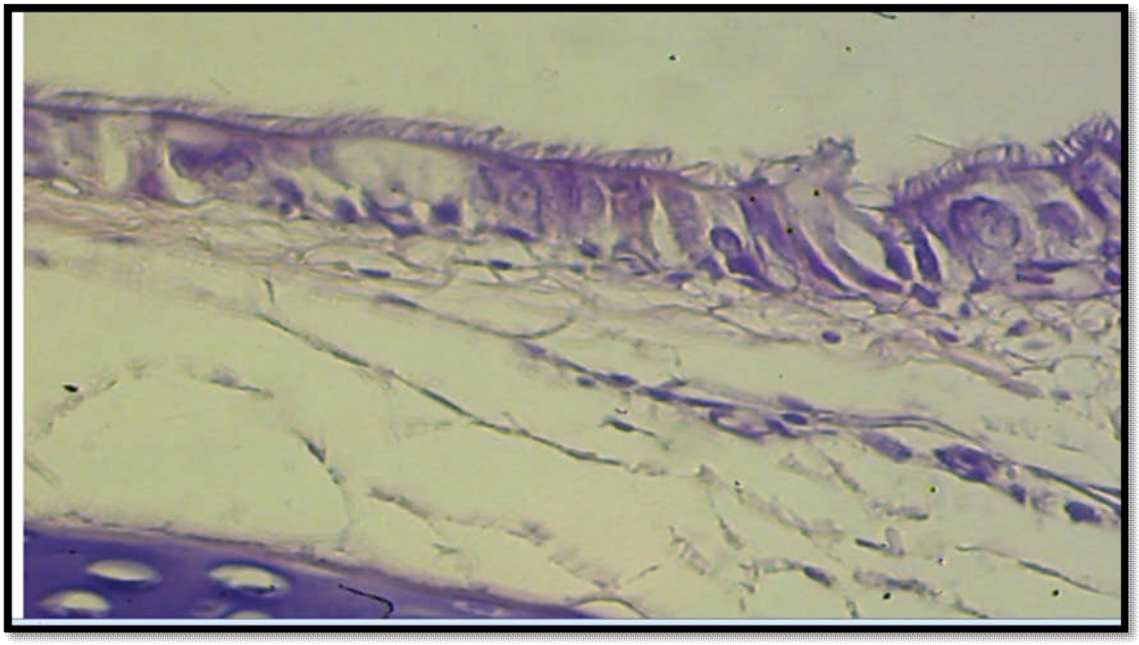


Fig: 9. Cross section of trachea of G2 control group shows the normal appearance of the epithelial layer, H&E stain, x 20

Bursa of Fabricius Lesions

experimentally infected G1chicks recoded some pathological lesion in bursa of fabricius after 9 and 14 days PI, include found some developmental stages of the parasite on the upper surface of the bursa and hypertrophy and hyperplasia of bursal epithelial cell, and, infiltration of mononuclear cells (fig: 10, 11) while there is no histopathological lesions in bursa of fabricius of G2 chicks (fig: 12).

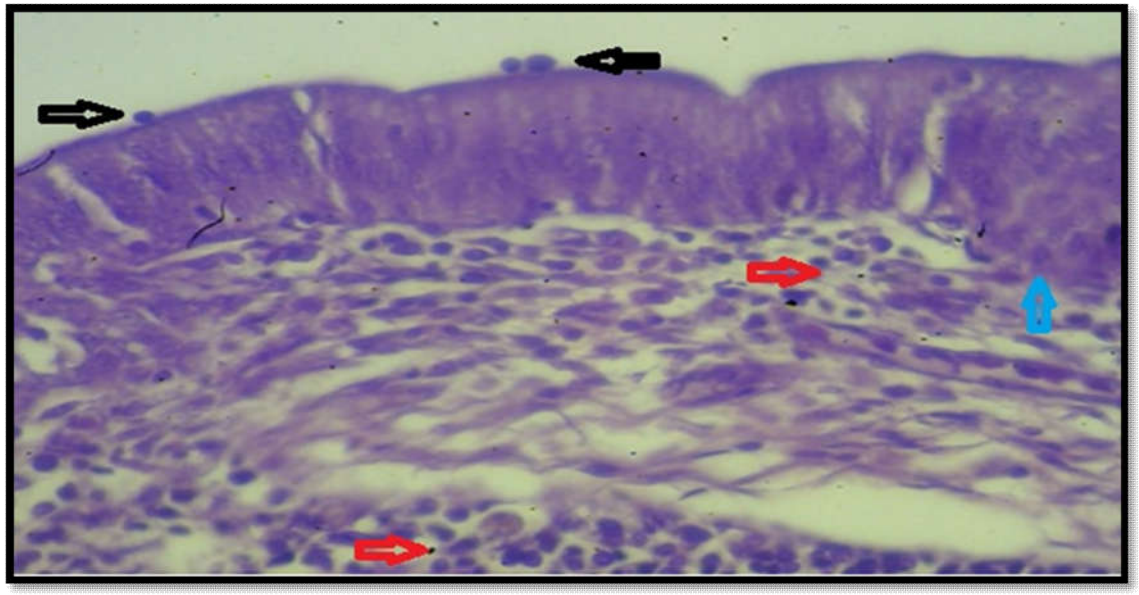


Fig: 10. Cross section of bursa of fabricius of G1 chicks after 9 days PI, seen some developmental stages of the parasite on the upper surface (Blue arrow), and hypertrophy and hyperplasia of bursal epithelial cell (Blue arrow), as well as infiltration of inflammatory cells (Red arrow), H&E stain X20.

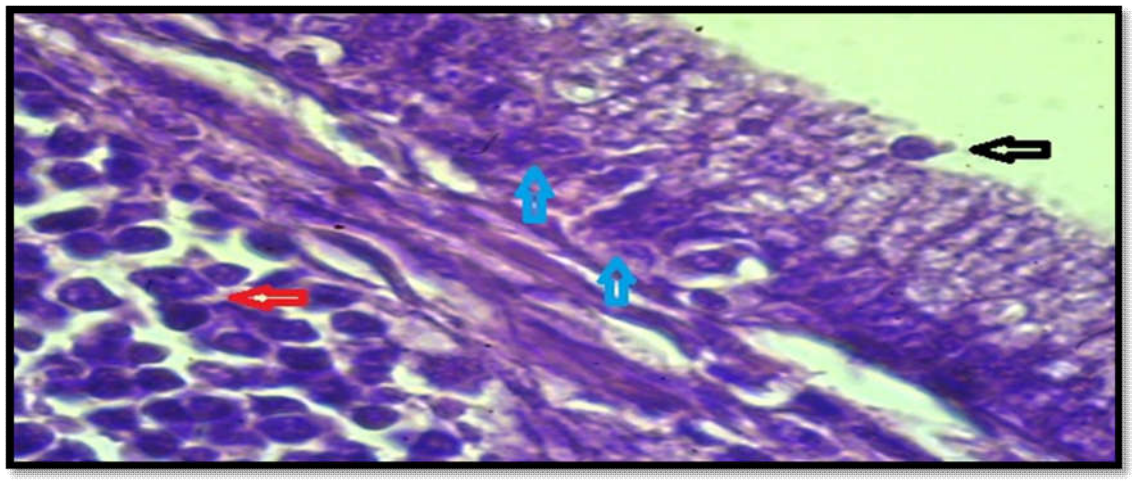


Fig: 11. Cross section of bursa of fabricius of G1 chicks after 14 days PI, seen some developmental stage of the parasite on the upper surface (Blue arrow), and hypertrophy and hyperplasia of bursal epithelial cell (Blue arrow), as well as infiltration of inflammatory cells (Red arrow), H&E stain X40.

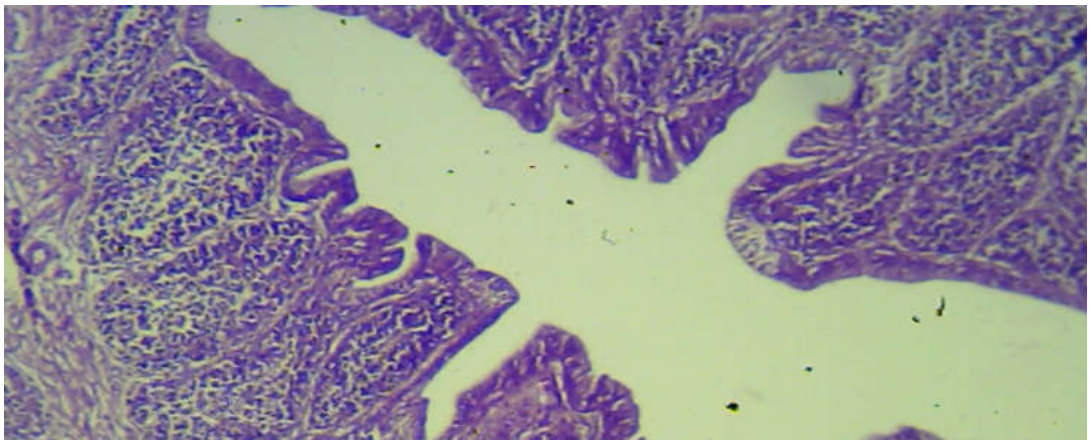


Fig: 12. Histopathological Cross section of the bursa of fabricius of G2 control group shows the normal appearance, and without presence of any developmental stages of the parasite H&E stain, x 10

DISCUSSION

The results of current study by using sheather's sugar solution the oocysts of *Cryptosporidium* appear transparent oval shapes, surrounded by a bright halo and contain undistinguishable four sporozoites, this result agreed with (2, 18, 19), who they found same characters of the parasite by using different traditional diagnostic methods.

The results of calibration of isolated oocysts, showed that the mean of measurement size of it was 6.2x 4.7 micrometers, this result of oocyst calibration *ryptosporidium* oocysts, showed that the measurement size of it was, which resemble the global size of *C.bailey*, (6.1x 4.5) micrometers. This result agrees with (1, 2, 15, 16, 20) who recorded same measurement size of *Cryptosporidium baileyi* oocysts in poultry .

Experimental Study

The results of experimentally infected broiler chicken chicks shows sever pathological changes in organs (intestine, trachea, and bursa of fabricius) of G1chicks, while without any pathological lesion in G2chicks or control group. The examination of their feces starting in the 3th day PI, and the shedding of oocysts in infected group G1, started in 4th days PI, while the first clinical signs, diarrhea, appear in the 6th days PI, than followed with other clinical signs such as, dullness, increased water

consumption, anorexia, while there is not found any clinical signs on the control group G2, The study recorded some gross lesion in the experiment infected groupG1 chicks, these post mortem changes included redness of intestinal wall with yellow feces also thickening in air sacs, while there is not found any gross lesion in control G2 group, this results agreed with (15, 16, 21, 22, 23), which recorded same clinical signs (dullness, increased water consumption, anorexia) , gross lesion (like redness and thickening of intestinal wall with yellow feces), and pathological lesion in intestine, trachea and bursa.

Histopathological lesion in organs of experimentally infected chicks:

Several experimentally infection studies (15, 16, 22, 23, 24) have been made by researchers to prove the isolated species of *Cryptosporidium* parasite through study the site of infection and pathological changes.

There was sever histopathological lesion shows in the result of study in the affected organs, include small intestine, trachea and bursa of fabricius, in G1chicks, while without any changes in chicks control group.

The intestinal histopathological lesions of the experimentally infected chickse showed severe epithelial distraction accompanied with widespread of necrosis leading 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 on the upper surface, as well as infiltration of sub mucosa layer with mononuclear cells (macrophages and plasma cells) in chicks of G1, after 9days and 14days PI , while there is no changes in intestine of G2 chicks.

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.(9, 15, 16, 21, 22, 23, 24,).

The results of tracheal cross section lesion of G1 chicks after 14 days is sever more than chicks killed after 9 days PI which include found of several developmental stages of the parasites appear oval or round structures on the upper surface of the epithelium, with deciliation of the mucous epithelium , also seen trachietis and

proliferated of epithelial layer, with mucinus materials on the surface of the tracheal epithelium and 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, with subcutaneous cell infiltration with plasma cells and heterophils as well as the proliferation of mononuclear cells, while there is no any pathological lesions in trachea of G2 chicks. This result agreed with.(9, 15, 16, 22, 23, 25, 26, 27) who they found same pathological lesion.

.

The result of experimentally infection on Bursa of Fabricius in G1chicks recoded some pathological lesion after 9 and 14 days PI, include found some developmental stages of the parasite on the upper surface of the bursa and hypertrophy and hyperplasia of bursal epithelial cell, and, infiltration of mononuclear cells, while there is no histopathological lesions in bursa of fabricius of G2 chicks. These pathological lesion agrees with Goodwin and Blaghum *et al*., (28) , Rhee *et al*., (29) in chicken and Alex and Marcelo, (9), in birds, Al-Zubaidi *et al*.,(16) and Al-Khayat, and Al-Zubaidi, (23) in broiler chicken chicks which recorded same lesion in bursa of fabricius of infected chicken and birds with cryptosporidiosis, include found of some developmental stages of the parasite on the upper surface of the bursa and hypertrophy and hyperplasia of bursa epithelial cell.

CONCLUSION

The current study proved that the isolated species of *Cryptosporidium* from slaughtered broiler chicken according to the global and local calibration or measurements of oocysts and the histopathological lesion in (intestine, trachea and bursa of fabricius) of natural infected chickens belong to the *Cryptosporidium baileyi*.

ACKNOWLEDGMENT

The authors are very grateful to Assistant Professor. Dr. Zainab. I. Ibrahim in department of pathology for the examination of histopathological sections.

REFERENCES

1-Xiao, L, Fayer R, Ryan, U. and Upton, S J. (2004). *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin Microbiol. Rev 17, (1):72 – 97.

2-Fayer R. and Xiao L. (2008). *Cryptosporidium* and Cryptosporidiosis. 2nd ed. CRC.Press. pp: 560.

3-Slavin D. (1955). *Cryptosporidium meleagridis* . (sp. Nov). J. Comp. Pathol 65, 262-266 . cited by (Fayer, 2010) .

4- Radostits O. M., Blood D. C. and Gay C. C. (1994). Cryptosporidiosis. In: Text Book of Vet. Med. Diseases of Cattle, Sheep, Pigs, Goats and Horses. 8th ed. W.B. Saunders Co. , London, pp : 1195-1199 .

5-Fayer, R.(2010). Taxonomy and Species delimitation in *Cryptosporidium.* Experimental Parasitology 124, (1): 90-97.

6-Al – Attar M. A. (1981) Factors affecting the pathogenesis of *Eimeria nicatrix* infections in ckickens. Ph.D. thesis .University of Guelph , Canada.

7-Silva D. C., Homem C., Nakamura A. A., Teixeira W. P., Perri S. V. and Meireles M. V. (2010). Physical, epidemiological, and molecular evaluation of infection by *Cryptosporidium galli* in Passeriformes . Parasitology Research 107, (2):271-277 .

8-Ryan U. (2010). *Cryptosporidium* in birds, fish and amphibians. Exp Parasitol 124, 113–120.

9-Alex A. N. and Marcelo V. M. (2015). *Cryptosporidium* infections in birds - a review. Braz. J. Vet. Parasitol 24, (3): 253-267.

10-Chermette R. and Boufassa Q S. (1988). Cryptosporidiosis a Cosmopital Disease in Animals and Man , 2nd ed . Office International Epizooties. France. pp: 1-122.

11-Thompson R. C., Olson M. E., Zhu G., Enomoto S., Mitchell S. A. and Hajjawi N. S. (2005). *Cryptosporidium* and Cryptosporidiosis. Adv. Parasitol 59, 77- 158

12-Al-Attar, A. and Abdul-Aziz T. (1985). Cryptosporidiosis of the bursa of Fabricius in broiler chickens. Iraq. J. Vet. Med 9, 49-53.

13-Ungar B. L. (1986) *Cryptosporidium spp.* And Cryptosporidiosis . Microbiol. Rev. 50 : 458 – 483.

14-Kim C.W. (1987) Chemotherapeutic effect of arprinocid in experimental Cryptosporidiosis . J. Parasitol. 73 : 663 – 666.

15-Al-Mahmood S. S. (2011). Experimental histopathological study of chicks infected with *Cryptosporidium baileyi* isolated from wild pigeos in Mosul. Iraqi Journal of Vet. Science 25, (1) 43-49.

16-AL-Zubaidi M. Th. S., Kadhim L. I., Ibrahim Z. I. and Al-Rikabi A. Sh. (2018). Incidence and Experimental Infection of *Cryptosporidium baileyi* in Chicken. Iraqi Journal of Agricultural Sciences. 49(2):269- 278

17-Luna L.G. (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd.ed. McGraw- Hill, New York, NY.

18-Kadir M.A. and El-Yassin S. (2002). Comparison of different laboratory methods for diagnosis of Cryptosporidium. Iraqi J. Vet. Med 26, (1): 153-158.

19-Al-Zubaidi M. Th. S. (2017). Molecular and microscopic detection of *Cryptosporidium spp*. International J of Science and Nature 8 (2) 372-376.

20-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.

21-Fayer R., Speer C. A. and Dubey J. P.(1990). General biology and histopathological changes caused by *Cryptosporidium baileyi*. CRC press, Florida. pp:1-30.

22-Blagburn B. L., Lindsay D. S, Giambrone J. J., Sundermann C. A. and Hoerr F. J. (2003). Experimental cryptosporidiosis in broiler chickens. Poult Sci 128, 442-449.

23-Al-Khayat K. Kh. K. and Al- Zubaidi M. Th. S. (2015). Some epidemiological study of *Cryptosporidium* spp. in broiler chickens in some areas of Karbala Province. Iraqi J Vet. Med 39, (1) 5-8.

24-Darabus G. H. (1997). Experimental studies of inter-and itraspecific transmission of *Cryptosporidium parvum* and *Cryptosporidium meleagridis* . Rev. Rom. Med. Vet 7, (2):155-160.

25-Goodwin M.A. (1989). Cryptosporidiosis in Birds-A review. Avian pathology.18:365-384.

26-Murakami S. M., Miyarna A., Ogawa J., Shimada O., and Nakane T. (2002). Occurrence of conjunctivitis, Sinusitis and upper region tracheitis in Japanese quail (*Coturnix coturnix japonica*), possibly caused by mycoplasma gallisepticum accompainied by *Cryptosporidium* sp. Infection. Avian Pathol. 31: 363-370.

27-Churria D. L, Sansalone A., Machuca B., Vigo H., Sguazza A., Origlia V., Piscopo A., Loyola A. and Petrucceli A. (2012). Tracheitis in a broiler chicken flock caused by duol infection with *Cryptosporidium* spp. (Apicomlexa: Cyptosporiidae) and Non-hemolytic *Ornithobacterium rhinotracheale*. Braz J. Vet. Pathol 5, (2) :89-93 .

28-Goodwin M. A. and Brown J. (1989). Light microscopic lesion associated with naturally occurring bursal Cryptosporidiosis in chickens. Avian Dis 33, 74-78.

29-Rahif R. H., Al-Kilani B. A. (2002). Prevalence of *Cryptosporidiium* oocysts water of white gold village of Baghdad, Iraqi Veterinary Medical Journal 26, (2): 44-55.