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Detection of natural cryptosporidial infection in slaughtered broiler chicken in local markets of Baghdad province

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Abstract

The present study aimed investigate the detection of cryptosporidiosis in 280 fecal samples from slaughtered broiler chicken carcasses in local markets in some areas of Baghdad city (AL-Aameriah, AL-Bayaa, AL-Doura Al –Kadhimiya, AL-Yarmok, AL-Turath, and Al-Shuala), during the period from the beginning of November 2017 to end of April 2018. Four laboratory diagnostic techniques used, flotation by Sheather's sugar solution, staining with Modified Zeihl-Neelsen stain, measuring of isolated *Cryptosporidium* oocysts (by ocular micrometer) and histopathological examination for natural infected samples to determine the type of *Cryptosporidium* species by measuring of oocystsand site of pathological lesion, and for conform that the isolated species of parasite from infected cases belong to the *C.baileyi*. The study demonstrated that the overall percentage of infection was 33.21%. The highest infection rate recorded in AL-Bayaa 47.5% (19/40), while the lowest rate recorded in AL-Yarmok 22.5% (8/40) with significant deference among regions. The results showed that the highest infection rate of *Cryptosporidium* parasite is occured in March and April which reach to46.80 %(22/47), 41 %(20/48) respectively. The results of calibration of isolated oocysts, showed that the mean of measurement size of *Cryptosporidium* oocyst was 6.2x 4.7 micrometers. The histopathological section made to the specimens of natural infected samples (small intestine and trachea) of slaughtered broiler chicken recoded the pathological lesions in these infected tissues. In conclusion: The total infection of *Cryptosporidium* in fecal samples was 33.21% (93/280).

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Introduction

Cryptosporidiosis is considered one of parasitic disease caused acute enteritis to the different types of animals and humans, this parasite describe for the first time by Jackson Clark in 1895 in intestinal mucosa of rat and called Swarm spores on it (xiao *et al.*, 2004). The infection occurs through ingestion of contaminated food and drinking water which contain mature oocysts. 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 (Fayer and Xiao, 2008).The importance of this parasite was increased in 1955 in poultry after the spread of it in turkey fields, and caused high morbidity and mortality rates in birds in Romania, and recorded high infection rates with economic losses, then began give attention to the classification of *Cryptosporidium*, and its species in the various vertebrates hosts (Slavin, 1955; Radostits *et al.*, 1994;Fayer, 2010).

Al-Attar and Abdul Aziz (1985) was firstly recorded the parasite in Iraq, in broiler chickens in Baghdad city with infection rate 8.8%, and isolate *Cryptosporidium* from the bursa of fabricius, without any clinical signs . Bird species, including poultry, are infected with three species of *Cryptosporidium*, *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 *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 (Fayer and Xiao, 2008; Silva *et al.*, 2010; Ryan,2010; Alex and Marcelo, 2015).

The current study was designed to estimate the prevalence of the parasite in broiler chickens which slaughtered in local markets in some areas of Baghdad province and determined the species of the parasite from calibration of oocysts and study the histopathological changes in site of infection (small intestine and trachea) in natural infected cases.

Materials and methods

Samples collection

Intestine and trachea were collected from (280) slaughtered chickens from local market from both sexes during the period from the beginning of November 2017 to end of Abril 2018, the study involved different region of Baghdad city (AL-Aameriah, AL-Bayaa, AL-Doura Al –Kadhimiya, AL-Yarmok, AL-Turath, and Al-Shuala). The fecal samples were tacked directly from intestine content in a clean plastic container (50ml size) and were tightly closed and given sequential numbers. All information for the animal included sex, 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

Microscopic examination of the feces was carried out. Each sample was divided into two parts: Thin smears were prepared and stained by Modified Ziehl-Neelsen stain.

And examined by flotation using Sheather's sugar solution.

Preparation of fecal smears and staining by the Modified Ziehl-Neelsen stain

Small amount of feces as matchstick head was mixed on a glass slide with a drop of distilled water and spread overall the slide, let to air-dry for 10 minutes**,** (taking into account numbering of the slide the number of sample itself).The smear was fixed in a concentrated methanol 99.5% for 5 minutes and let to dry. Smear was immersed in the red strong carbol-fuchsin for 3 minutes. Slide was washed thoroughly in tap water. The red color was decolorized by acidic alcohol for 30 seconds, and then the slide rinsed in tap water. Methylene blue was used as a counter stain

for 2 minutes, then washed in tap water and let to dry. The slide examined for the presence of oocysts by scanning using the ×40 objective lens of a bright-field microscope and then the oil immersion objective lens ×100. (Beaver & Jung, 1985).

Floatation using Sheather's sugar solution

Fecal samples (3-5 grams) 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 (Chermette and Boufassa, 1988).

Ocular micrometer

This method was used to determine the oocysts calibration or measurements according to (Thompson *et al.*, 2005).

Histopathological Examination of natural infected cases

Study histopathological section made to the samples of intestine and trachea of infected and none infected cases from the survey samples to confirm the presence of the parasite with it is pathological lesion in infected tissues. (Luna, 1968).

Statistical analysis

The Chi-square test was used for the comparison between the results.

Differences were considered statistically significant at $P<0.05$ (Snedecor and Cochran, 1989).

Results

Effect of areas, months, and sex on infection rate

The results of this study showed no significant differences in the infection rates of *Cryptosporidium* in slaughtered broiler chicken among the areas of Baghdad city.

The percentage of total infection of *Cryptosporidium* in fecal samples was 33.21% (93/280), the highest rate recorded in AL-Bayaa 47.5% (19/40) while the lowest rate recorded in AL-Yarmok 22.5% (8/40) (Table 1).

Table 1. Rate of infection with *Cryptosporidium spp.*according to the areas.

Areas	No. of Samples examined	No. Positive	Infection Rate %
AL-Aameriah	40	13	32.5
AL-Bayaa	40	19	47.5
AL-Doura	40	13	32.5
AL-Kadhmeyah	40	14	35
AL-Yarmok	40	8	22.5
AL-Turath	40	10	25
AL-Shaula	40	16	40
Total	280	93	33.21
Chi square value	8.95		
P	0.17		

According to the months of study the results shows that the highest infection rate of *Cryptosporidium* parasite occur in March and April which reach 46.80%(22/47), 41%(20/48) respectively, while the lowest infection rate found in December 23.91% (11/46), with no significant difference (Table 2).

Table 2. Rate of infection with *Cryptosporidium spp.* according to the months.

months of study	No. of Samples examined	No. Positive	Infection Rate %
November	48	13	27.08
December	46	11	23.91
January	45	12	26.66
February	46	15	32.60
March	47	22	46.80
April	48	20	41.66
Total	280	93	33.21
Chi square value	8.94		
P	0.11		

The result of study according to the sex not recorded any significant difference in infection rate of *Cryptosporidium* between male and female, 33.13 % (56/169), 33.33 %(37/111) respectively (Table 3).

Table 3. Rate of infection with *Cryptosporidium spp.* according to the sex.

Sex	No. of Samples examined	No. Positive	Infection Rate %
Male	169	56	33.13%
Female	111	37	33.33%
Total	280	93	33.21%
Chi square value	0.001		
P	0.97		

The result of study recorded that the shape and calibration of *Cryptosporidium* oocyst, using sheather's sugar solution appear the transparent oval shapes, surrounded by a bright halo and contain undistinguishable four sporozoites (Fig.1).

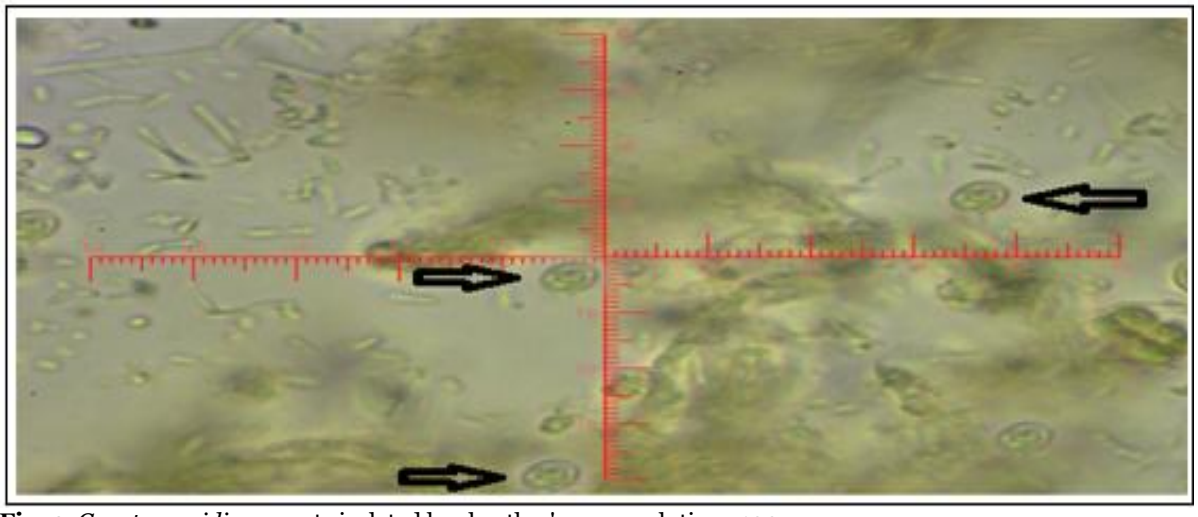

Fig. 1. *Cryptosporidim* oocysts isolated by sheather's sugar solution x100.

While by using MZN stain the oocysts of parasite appeared glowing red, with blue background according to the opposite color used (Fig. 2). The results of calibration of isolated oocysts, showed that the mean of measurement size of parasite was 6.2x 4.7 micrometers (Fig. 3).

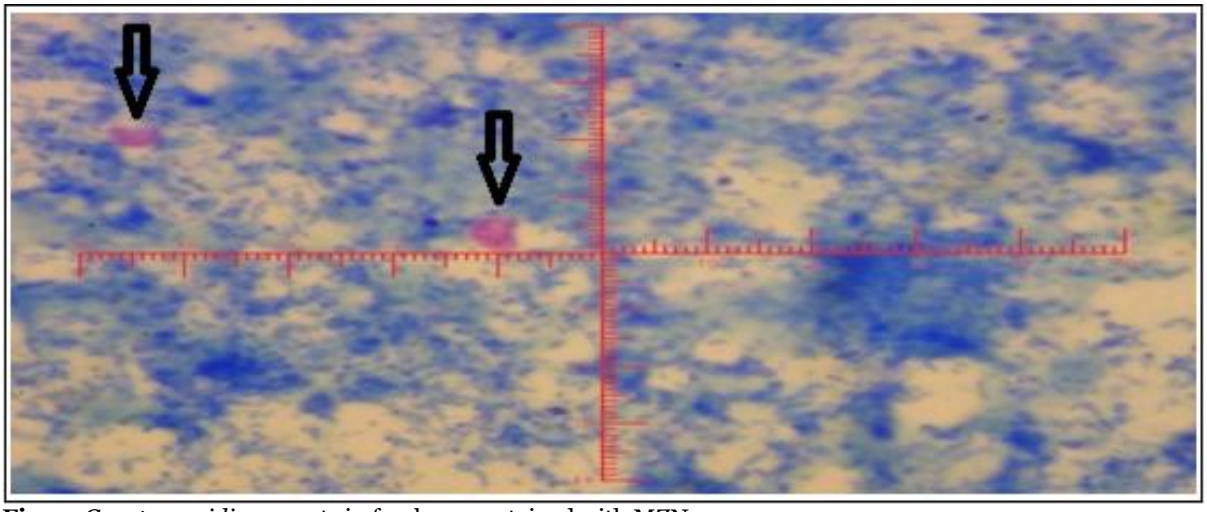

Fig. 2. *Cryptosporidim* oocysts in fecal smear stained with MZN x100.

Histopathological Examination of natural infected cases

Histopathological section of intestine and trachea specimens of survey slaughtered chickens samples, made to confirm the presence of the parasite and study the pathological lesion, which caused by parasite. The result of intestine sections shows presence of developmental stages of the parasite on the upper surface, with severe necrosis and degeneration of mucosal epithelium, (Fig. 4) with infiltration of inflammatory cells in lamina properia extended to submucosa and accumulation of necrotic debris in lumen which compared with normal tissue of intestine from villi covered with columnar epithelium (Fig. 5).

While the sections of trachea of infected specimens showed appearance of round or oval structures on the upper surface of the epithelium which considered the parasite stages, with infiltration of inflammatory cells and distraction of the epithelial surface, while in non-infected cases there is no any lesion in Fig.6 and Fig.7 respectively.

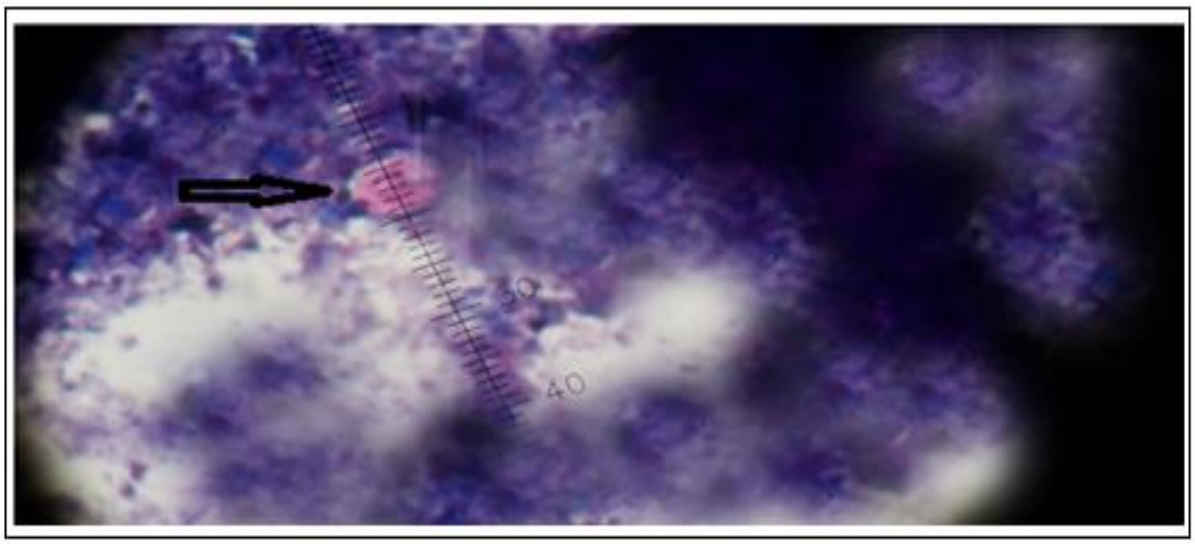

Fig. 3. *Cryptosporidim* oocysts calibrated with ocular micrometer x100.

Discussion

The results of the present study recoded that the total infection rate was33.21% in the slaughtered broiler chicken suffered from cryptosporidiosis. This result agreed with the results obtained by Al Bayati (2002) in Iraq, which found that the proportion of parasitic

infection in broiler chickens in Baghdad was 21.82%, and approached the study of Kichaw *et al*., (1996) in Morocco, which found 24% of chicken infected with the parasite, and also approached with Papadopoulou *et al*. (1988), which recorded a rate of infection in Greece reached to 24.3% in broiler chickens. The results were consistent with what was found by Darabus (1997) in Romania, which recorded 22.5% in broiler chickens and with the results of Shemshadi *et al*., (2010), which recorded 23.8% of broiler chickens infected with cryptosporidiosis in Iran. While the result disagreed with Al-Attar and Abdul Aziz (1985) in Iraq, who recorded 8.8% in Baghdad and Kucukerden *et al*., (1999) in Turkey who found 4.4% of broiler chickens infected.

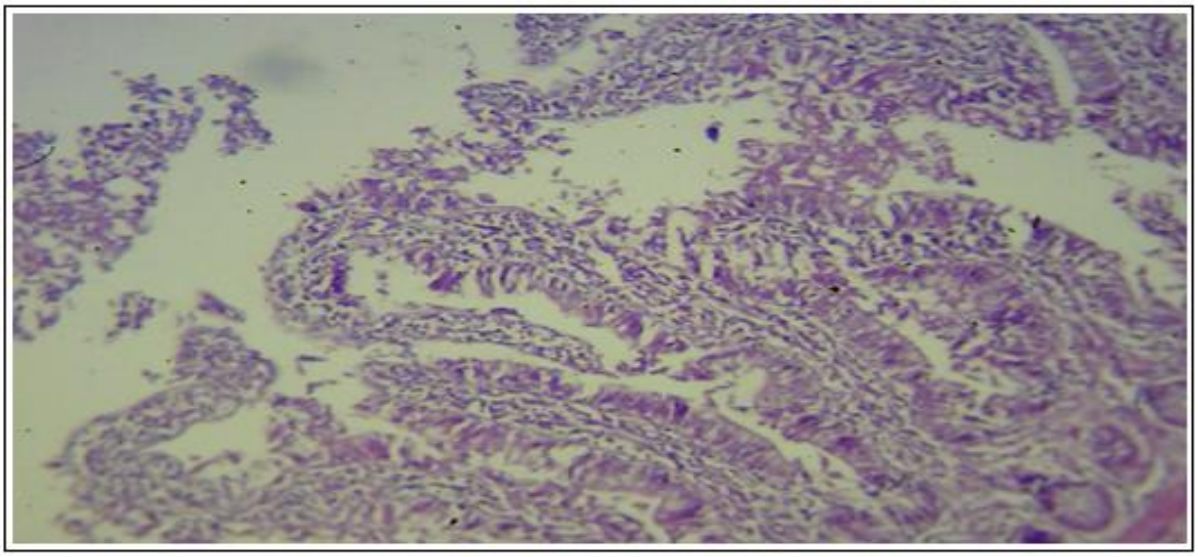

Fig. 4. Intestinal section of infected case with *Cryptosporidium* H&E stain X40.

The variation in incidence of Cryptosporidiosis in broiler chickens in these studies, may be attributed tomany 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) (Fayer and Xiao, 2008; Al-Zubaidi *et al*., 2018).

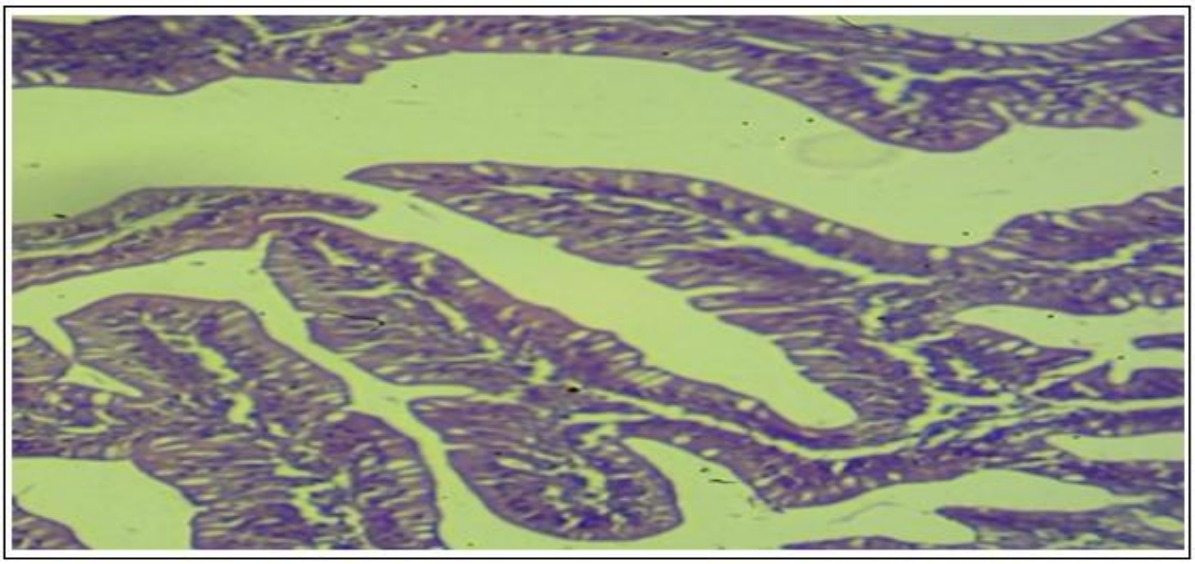

Fig. 5. Normal section of survey specimens of intestine(X40), without any pathological changes H & E stain.

The result of this study showed a significant difference in infection rate according to the months, The results shows that the highest infection rate of *Cryptosporidium* parasite occur in March and April which reach 46.80 %(22/47), 41 %(20/48) respectively, that's agreed with researcher Rongjun *et al.* (2010) who recorded the highest rate of infection in the spring months 15.6% and noted a significant decline in summer and autumn reached 2.0%, also agrees with Rahif and Al-Kilaniin,(2002)in Baghdad who reported highest number of *Cryptosporidium* oocysts presence in water and spring months, and the lowest number in the summer months. While the results did not agreed with Goodwin and Brown (1989), who found that the highest rate of infection was in the summer and the lowest in winter because the high exposure of broilers chicken to stress due to high temperature and humidity, this interpretation is contrary to the reality of local education in terms of providing cooling and Suitable ventilation in typical breeding halls.

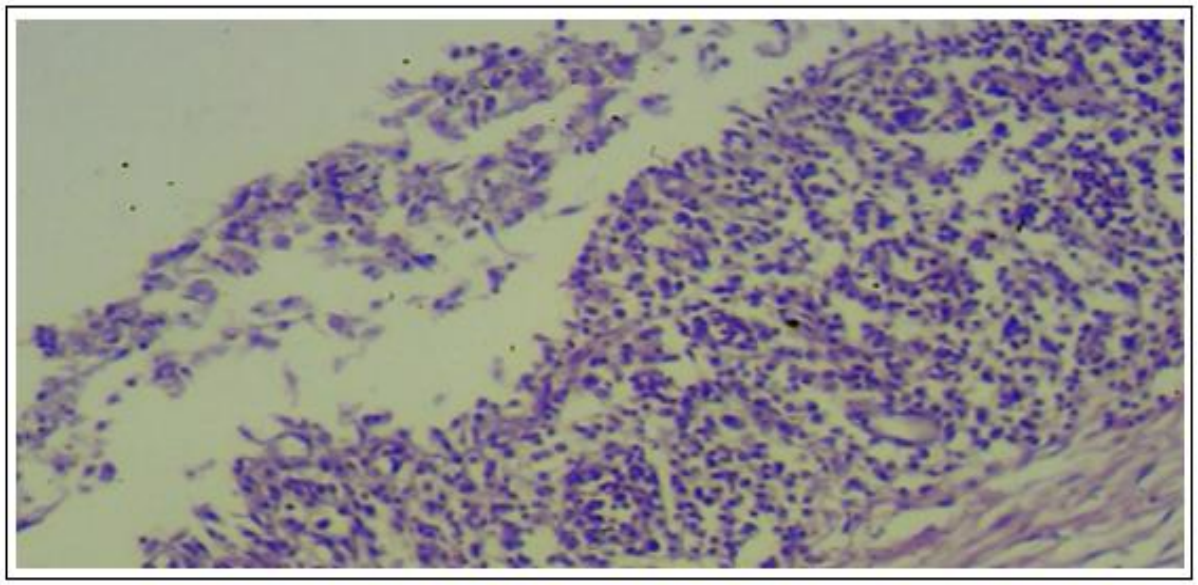

Fig. 6. Tracheal section of infected case with *Cryptosporidium* H&E stain X40.

The results of study in male and female 33.13 %(56/169), 33.33 %(37/111) respectively, not recorded any significance difference in infection rate with cryptosporidiosis in slaughtered broiler chickens, this result agreed with several researchers who not recoded difference in infection rate between male and female due to the exposure to the same condition factors like high temperature and humidity (Casemore, 2000; Blagburn *et al.*, 2003).

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, while by using MZN stain the parasite take red to pink color, with blue background according to the opposite color used (methylen blue stain). This result agreed with (Kadir and Yassin, 2002; Fayer and Xiao, 2008; Al-Zubaidi, 2017), 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 calibration of isolated *Cryptosporidium* oocysts, showed that the measurement size of it was 6.1x 4.5 micrometers, which resemble the global size of *C.bailey*. This result agrees with (Xiao *et al*, 2004 ;Fayer and Xiao, 2008; Al-Mahmood, 2011; Al-Bakri, 2012 and Al-Zubaidi *et al.*, 2018) who recorded same measurement size of *Cryptosporidium baileyi* oocysts in poultry .

Histopathological section of intestine and trachea specimens of survey infected and non-infected slaughtered chickens samples, the study recorded presence of the parasite and several pathological lesion, include presence of parasite, with complete destruction of ciliated mucosal epithelium, 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 intestinal epithelium, while the sections of trachea of infected specimens showed, the appearance of round or oval structures on the upper surface of the epithelium which considered the parasite developmental stages, with infiltration of inflammatory and distraction of the epithelial surface, while in non-infected cases there is no any lesion.

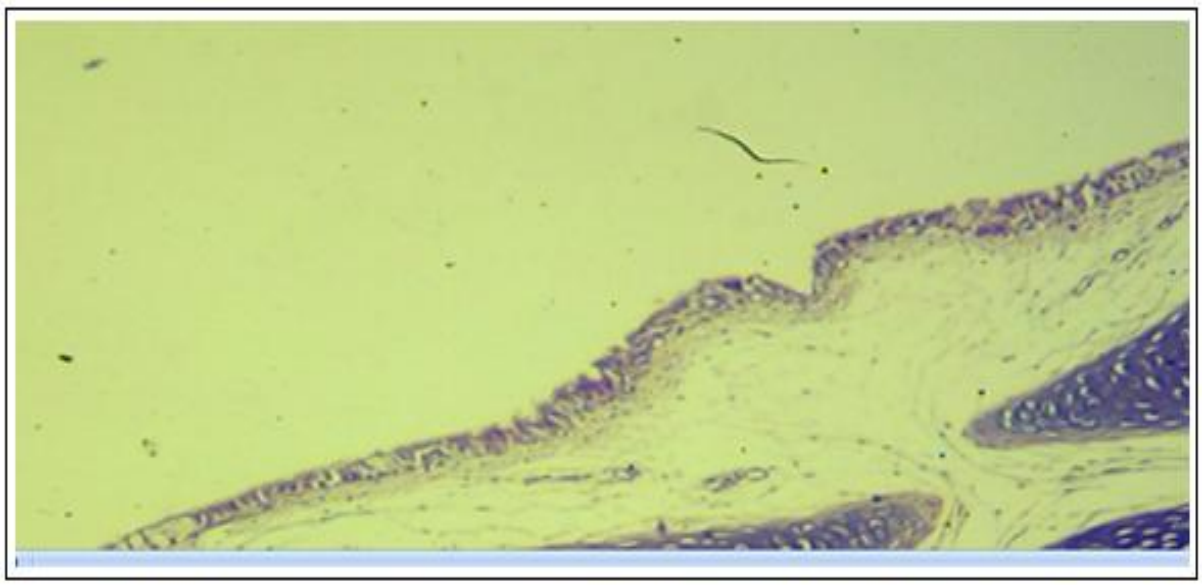

Fig. 7. Tracheal section of normal or not infected case of survey specimens, without any pathological changes H&E stain(x20).

This results agreed with (Itakura *et al.*, 1984; Nakamura and Abe, 1988;Goodwin, 1989; Fayer *et al.*, 1990; Murakami *et al.*, 2002; Al-Mahmood, 2011; Churria *et al.*, 2012;Al-Khayatand Al- Zubaidi, 2015; Al-Zubaidi *et al.*, 2018) who they recoded same pathological lesion in intestine and trachea of experimentally infected of broiler chickens chicks with *C. baileyi*, and recorded several pathological lesion, include presence of developmental stages of the parasite on the upper surface of the epithelial layer of intestine and trachea, with deciliation of the mucous epithelium, severe necrosis accompanied by debris and infiltration of inflammatory cells as well as the presence of mucosal hyperplasia.

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 (intestineand trachea) of natural infected chickens belong to the *Cryptosporidium baileyi*.

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References

Alex AN, Marcelo VM. 2015. Cryptosporidium infections in birds - a review. Brazilian Journal of Veterinary Parasitology **24(3),** 253-267.

Al-Attar A, Abdul-Aziz T. 1985. Cryptosporidiosis of the bursa of Fabricius in broiler chickens. Iraqi Journal of Veterinary Medicine **9,** 49-53.

Al-Bakri HS. 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.

Al-Bayati HS. 2002. Study of cryptosporidiosis spp. In the fields and slaughter house and its relationship with the workers. M.Sc. Faculty of Veterinary Medicine. University of Baghdad, Iraq.

Al-Khayat KKHK, Al-Zubaidi MTHS. 2015. Some epidemio logical study of Cryptosporidium spp. in broiler chickens in some areas of Karbala Province. Iraqi Journal of Veterinary Medicine **39(1),** 5-8.

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

Al-Zubaidi MTHS. 2017. Molecular and microscopic detection of Cryptosporidium spp. International Journal of Science and Nature **8(2),** 372-376.

AL-Zubaidi MTHS, Kadhim LI, Ibrahim ZI, Al-Rikabi ASH. 2018. Incidence and Experimental Infection of Cryptosporidium baileyi in Chicken. Iraqi Journal of Agricultural Sciences **49(2),** 269-278.

Beaver PC, Jung RC. 1985. Animal Agents and Vectors of Human Disease.5th ed. Lea and Febiger, Philadelphia p 249.

Blagburn BL, Lindsay DS, Giambrone JJ, Sundermann CA, Hoerr FJ. 2003. Experimental cryptosporidiosis in broiler chickens. Poultry Science **128,** 442-449.

Casemore DP. 2000. Human Cryptosporidiosis: Clinical aspects, epidemiology and control. Proc. R. Coll. Physicians Edinb **30**, 287-293.

Chermette R, Boufassa QS. 1988. Cryptosporidiosis a Cosmopital Disease in Animals and Man , 2nd ed. Office International Epizooties. France, p 1- 122.

Churria DL, Sansalone A, Machuca B, Vigo H, Sguazza A, Origlia V, Piscopo A, Loyola A, Petrucceli A. 2012. Tracheitis in a broiler chicken flock caused by duolinfection with Cryptosporidium spp. (Apicomlexa: Cyptosporiidae) and Non-hemolytic Ornithobacterium rhinotracheale. Brazilian Journal Veterinary Pathology **5(2)**, 89-93.

Darabus GH. 1997. Experimental studies of inter- and itraspecific transmission of Cryptosporidium parvum and Cryptosporidium meleagridis. Revista Română de Medicină Veterinara **7(2),** 155-160.

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

Fayer R, Xiao L. 2008. Cryptosporidium and Cryptosporidiosis.2nd ed. CRC. Press. 560.

Fayer R, Speer CA, Dubey JP. 1990. General biology and histopathological changes caused by Cryptosporidium baileyi. CRC press, Florida., 1-30.

Goodwin MA. 1989. Cryptosporidiosis in Birds-A review. Avian Pathology **18,** 365-384.

Goodwin MA, Brown J. 1989. Light microscopic lesion associated with naturally occurring bursal Cryptosporidiosis in chickens. Avian Diseases **33**, 74-78.

Itakura C, Goryo M, Umemura T. 1984. Cryptosporidial infection in chickens. Avian Pathology **13(3),** 487-499.

Kadir MA, El-Yassin S. 2002.Comparison of different laboratory methods for diagnosis of Cryptosporidium. Iraqi Journal of Veterinary Science **26(1),** 153-158.

Kichaw F, Saghir F, EL-Hamidi M. 1996. Infection naturally by Cryptosporidium spp. Chezlepoulet de chair au Maroco. Avian Pathology **25,** 103-111.

Kucukerden N, Ozturk G, Angin M. 1999. The prevalence of Cryptosporidiosis in broilers at Elazig district. Turkish Journal of Veterinary and Animal Science **21**, 153-156.

Luna LG. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd.ed. McGraw- Hill, New York, NY.

Murakami SM, Miyarna A, Ogawa J, Shimada O, Nakane T. 2002. Occurrence of conjunctivitis, Sinusitis and upper region tracheitis in Japanese quail (Coturnix coturnix japonica), possibly caused by mycoplasma galli septicumac compainied by Cryptosporidium sp. Infection. Avian Pathology **31,** 363-370.

Nakamura K, Abe F. 1988. Respiratory (Especially pulmonary) and urinary infections of Cryptosporidium in layer chickens. Avian Pathology **17(3),** 703-711.

Papadopoulou C, Xylouri E, Zisides N. 1988. Cryptosporidium infection in broiler chickens in Grace. Avian Diseases **3,** 842-843.

Radostits OM, Blood DC, Gay CC. 1994. Cryptosporidiosis. In: Text Book of Vet. Med. Diseases of Cattle, Sheep, Pigs, Goats and Horses. 8th ed. W.B. Saunders Co. , London, p 1195-1199.

Rahif RH, Al-Kilani BA. 2002. Prevalence of Cryptosporidiium oocysts water of white gold village of Baghdad, Iraqi Veterinary Medical Journal **26(2),** 44-55.

Rongjun W, Fuchun J, Yanping S, Qunshan H, Jingjing Z, Fang W, Changshen N, Longxian Z, Lihua X. 2010. Large-scale survey of Cryptosporidium spp. in chickens and Pekin ducks (Anasplatyrhynchos) in Henan, China: prevalence and molecular characterization, Avian Pathology **39(6),** 447-451.

Ryan U. 2010. Cryptosporidiumin birds, fish and amphibians. Experimental Parasitology **124,** 113–120.

Shemshadi B, Bahadori SR, Mozafari A. 2010. Study on cryptosporidiosis incidence in broilers in Garmsar region, Iran. Comparative Clinical Pathology.
http://dx.doi.org/10.1007/s00580-010-0970-0

Silva DC, Homem C, Nakamura AA, Teixeira WP, Perri SV, Meireles MV. 2010. Physical, epidemiological, and molecular evaluation of infection by Cryptosporidium galliin Passeriformes. Parasitology Research **107(2),** 271-277.

Slavin D. 1955. cryptosporidium meleagridis. (sp. Nov). Journal of Comparative Patho**logy 65,**262-266 .cited by (Fayer, 2010).

Snedecor GW, Cochran WG. 1989. Statistical Methods, Eighth Edition, Iowa State University Press. p 503.

Thompson RC, Olson ME, Zhu G, Enomoto S, Mitchell SA, Hajjawi NS. 2005. Cryptosporidium and Cryptosporidiosis. Advances in Parasitology**59**, 77-158.

Xiao L, Fayer R, Ryan U, Upton SJ. 2004. Cryptosporidium taxonomy: recent advances and implications for public health. Clinical Microbiology Review **17(1),** 72 –97.