SOME PATHODIAGNOSTIC OBSERVATIONS ON CRYPTOSPORIDIOSIS OF CHICKENS IN MOSUL CITY, IRAQ

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ABSTRACT

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This study was assessed to detect and diagnose cryptosporidium parasite of chickens (Galluas gallus) using three especial stains (Modified Zeihl-Neelsen, Giemsa's, and Saffranin methylene blue stains) by direct smears. In this study (80) fecal droppings were collected from chickens at different ayes and sexes used chickens suffered from emaciation non bloody diarrhea raffled feat theirs and respiratory disorders with nosal discharge. Accurate diagnosis was optioned using Ziehl-Nelsecnstain (62.27) cereyiored to saffranin methylene blue stains 20% and Giemsa stain 15%. Scrapings and imprints of small intestine trachea and Bursa of fabricous from (20) necropsied birds, revealed 18.3% infection in the small intestine, 8.4% in trachea and 1.6% in Bursa of Fabricous. The results of microscopic examination of cytological smears were more sensitive and accurate for oocyst of cryptosporidia by modified Ziehl-Nelseen stain, than by Saffranin methylene blue stains, and Giemsa stain. For study of histopathological changes of this protozoa in infected tissues 10 samples were collected from small intestine, trachea and Bursa of Fabricous from necropsed birds and fixed, stained with Haematoxylene – Eosine stain, the main pathologic changes of small intestine were atrophic misshapen villi and with infiltration of Lamina properia with inflammatory cells. In Trachea mucinous degeneration, deciliation and sloughing. In Bursa of Fabricous the changes involve depletion of lymphocyte. Microscopic examination of 10 samples from small intestine trachea and Bursa showed that the small intestine were atrophic with misshapen vulli and cellular infection of the commproperia muanous degeneration defilation and sloughing of tracheal epith.

Key words: *Crypto, cryptosporidiosis, oocyst, modified Ziehl-Nelseen*

INTRODUCTION

Cryptosporidium is enteric protozoan pathogen, smallest of any coccidian parasite, (phylum Apicomlexa, genus Cryptosporidium) that infect human, domestic animals and birds, oocyst size (4-8 X 5-6 µm) (Lindsay and Blagburn, 1986).

Cryptosporidium species that have been described in birds include C.meleagridis C.baileyi, C.tyzerri and C.galli(Fayer and Unger, 1986).

Avian Cryptosporidiosis can manifest as respiratory and intestinal diseases (Goodwin and Brown, 1994). Members of oocysts are small; spheroid to void protozoan parasites. C.baileyi is probably the most common avian Cryptosporidium species of chickens, turkeys, ducks, quails, ostriches and cockatials.

C.baileyi, which is the most prevalent species in poultry, generally infect the respiratory tract, resulting in coughing sneezing and mucoid discharge in respiratory tract. C.meleagridias infects the intestines where it cause mild to sever enteritis, diarrhea, dehydration, weight loss and weakness. Young birds appear more susceptible to infections. (Ley and Guy, 1987).

Cryptosporidium infect, grow and replicate in the gastrointestinal and respiratory epith. Infections by Cryptosporidium are now known to be wide spread and frequently associated with waterborne illness and zoonosis. (Current *et al.*, 1986).

Infections with Cryptosporidium are routinely diagnosed by fecal examinations (direct smear) for the presence of oocysts using modified acid fast stain(Ziehl – Neelsen Stain) or other staines, oocysts are shed already sporulated and contain 4 sporozoites(Henoviksen and Pohlenz; 1981) Also smears of Mucosal scraping and multiple imprints for cytological evaluation, can be beneficial for accurate diagnosis.

Cryptosporidium Spp. are coccidian parasite that inhabit the microvillous borders of epithelial cells, lining the trachea, nasal cavity, small intestine and Bursa of Fabricous. It develops in an intracellular extracytopalsmic locations in the apical surface of epithelial cells with autoinfection cycle (Goodwin, 1989).

Cryptosporidium Spp. are prevalent in domesticated, caged, and wild birds in Malaysia the prevalence rate among chickens was (3.4%) (Yal and Muhamat, 2007), in Georgia (6.8%) (Goodwin and Brown, 1996), in Nigeria in birds (15.2%) (Umar, 2007), in Iran the incidence of Cryptosporidium in broiler was (8.2%) (Banani and Dadrass, 2000) and in Egypt in (1997) Turkey poulets (46.7%) (Entessar and Sahlab, 1997). In North Carolina Cryptosporidium spp. oocysts were found in the feces of 27.3% of broilers (Serter and Varga, 2000).

MATERIALS and METHODS

Sample Collection:

A total of (80) fecal droppings from native breed chickens of different ages, sexes were collected in Mosul city from April 2013 to February 2014 [Flocks, houses rearing and markets] and from poultry farm of college of Agriculture and Teaching hospital of the Veterinary College (Mosul).

Fecal smears were stained with (Safranin-Methylene blue, Gimsa and Modified Ziehl- Neelson stain) for identification of oocysts of cryrptosporidia in fecal droppings smears.

Scrapings and Impression smears were prepared from (20) small intestines of diseased chicken at post-mortem examination, Also Bursa of Fabricous and Trachea of suspected birds were stained and examined for cytology, by modified Ziehl-Neelsen stain and other stains.

Histopathological samples (10) from small intestine and respiratory tract and Bursa of Fabricous from suspected chickens were collected and fixed in 10% formalin.

Chickens suffering from non- bloody diarrhea, emaciation rough feathers and resp. disorders (sinusitis) were necropesed, gross lesions were repovted and smears were prepared from fecal dropping and from mucosal scrapings, impression smears of Bursa of Fabricous and tracheal

Methodology:

1- Fecal smears: 3 smears from each fecal droppings was prepared on three different glass slides.
2- Staining procedure: three different staining methods were employed in this study, Safranin – methylen blue stain Giemsa stain, modified Ziehl -Neelson stain technique (Casemor and Armstrong, 1985).
3- Scraping and Impressions smears of small intestine and Bursa of fabricious and trachea, stained with M.Z.N. stain, and other two stains were examined using oil immersion lens (100X) (Arrowood, 1997).
4- From resp. tract Bursa of Fabricous and small intestine fixed in 10% formalin and processed in the lab. Stained with HE stain for lesions or detection of the organism (oocysts) (Hoerr *et al.*, 1987)
5- Ocular micrometer for calibration of detected oocysts was used.

RESULTS

Microscopic detection of Cryptosporidium oocyst spp from (80) fecal droppings of chickens with different ages suffered from non-bloody diarrhea, entertis, emaciation ruffled feathers were presented in table (1) and Fig (1), (2), and (3) and (7).

Of 80 fecal droppings examined eryptosporidium oocyst were reported in 20.4%. 26.2%, 20% and 15% were positive when modified ziehl-Neelen saffranin methylene blue and Giemsa stained were used respictivally Oocyst size was 6.4 -3.8µ.

Table 1: Comparison of different staining methods in diagnosis of Cryptosporidium oosysts. Using direct method.

Types of stain \ No.	Direct exam		
	No. of sample tested	NO of (+)	% of (+)
Safranin-methylene-blue stain	80	16	20
Mod. Ziehl-Neelen stain	80	21	26.2
Giemsa stain	80	12	15
Oocyst size	4.6-6.8µ		
Oocyst No. / Field	0-4		
Total slides R (smcar) examined	240	49	20.4

Significant at $P<0.05$

Table 2: Results of Cytological examination for cryptosporidia oocyst (No. of samples 20)

Tissue / Stains	Small intestine		Trachea		Bursa of Fabricous	
	No +	%	No +	%	No +	%
Modified Ziel- Neelsen stain (205)	5	25	2	10	1	5
Saffeanin Methylene Blue (205)	3	15	2	10	0	0
Giems' stain (205)	3	15	1	5	0	0
Total	11		5		1	
Oocysts size	3.8 x 5.8 µ					
Oocysts No.	0.3		0-1		0-1	
Total	11	%18.3	5	%8.4	1	%1.6

Fig. 1: Oocysts of Cryptosporidia from feces stained with Modified Ziehl-Neelsen Stain 1000X

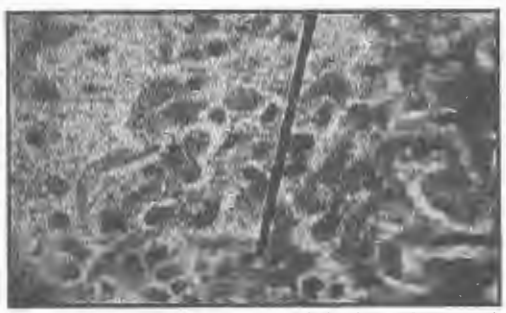

Fig. 2: Oocysts of Cryptosporidia from feces stained with Saffranin Methylene Blue stain 1000X

Fig. 3: Oocysts of Cryptosporidia from feces stained with Giemsa Stain 1000X

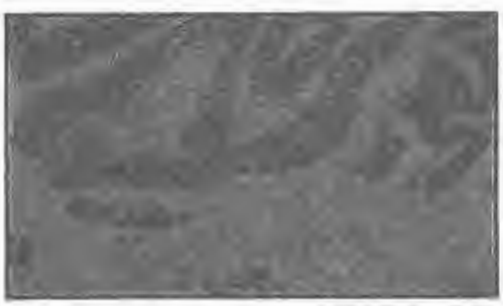

Fig. 4: Histopathological section of small intestine of chicken stained with Haematoxylene-Eosin stain 1000X

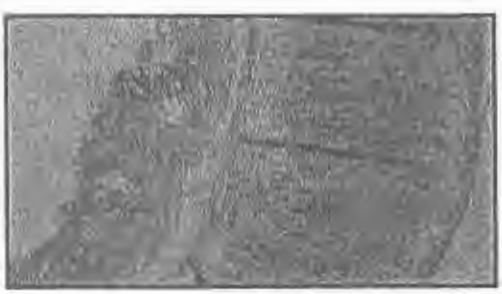

Fig. 5: Histopathological section of Trachea of chicken stained with Haematoxylene-Eosin stain 1000X

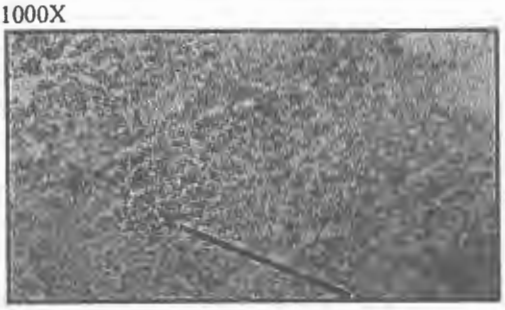

Fig. 6: Histopathological section of Bursa of Fabricous chicken stained with Haematoxylene-Eosin stain 1000X

Fig. 7: Oocyst of Cryptosporidia from fecal dropping stained by M.Z.N.

Putative cryptosporidia. Sp. Oocysts appeareet as bright rose-pink smooth spheres (5.6±2μ) on green dark ground by Modified Ziehl-Nelseen stain. Fig (1).

In Giemsa staining oocysts appeared dark-blue bodies Fig(2). And in saffranine mythelen blue stain the oocyst appear bluish – red Fig (3).

Scrapings and impression smears from small intestain, Trachea and Bursa of Fabrisues of necropsied birds stained with the three used stain showed 18.3%, 8.4% and 1.6% positive oocyst respectively.

Histopathology:
Atrophic and misshapen villi were reported in all segments of small intestine. The Lamina properia was infiltrated by sheets of inflammatory cells (heterophils,macrophagers, lymphocytes and plasma cells). Small basophilic spherules 5μm diameters Cryptosporidium sp.) was embedded within the microvillous border of enterocytes. An obvious signs of chronic enteritis, mucinous degeneration are noticed at intestinal villia with presence of cyrptosporidia oocyst (Fig. 4).

Trachea: Mucinous degeneration and increase in the number of goblet cell stages with mild deciliation and sloughings has been noticed (Fig. 5).

Bursa of Fabricous: Mild changes of Bursal tissue characterized by mild depletion of lymphocyte. accompanied with attachment of the plical epithelium in which the parasite is seen (Fig.6).

DISCUSSION

The purpose of this study was to compare the accuracy of three staining techniques used in diagnosis and also cytology from (scrapings and imprints, smears for diagnosis of cryprosporidia oocyst of chickens). Despite the important of Avian Cryptosporidiosis as zoonotic potential worldwide, there are few studies that have tried to identify this protozoan in poultry. (Arrwood, 1997).

The overall rate of infection with cryptosporidium Spp. oocysts in domestic chickens was (20.4%), this is similar to reports of (Itakura *et al.*, 1985). Modified Zeihl-Neelsen stain method was the best (26.2%) for detection of cryptosporidium Oocysts compared Saffranen-methylen blue (20%) and Giemsa stain (15 %). (This is significant at $P<0.05$). The number of samples is to small to gudge the sensitivity of this three stains.

This was in agreement with the results mentioned by (Henriksen and Pohlenz, 1981). table (1), (2).

In stained fresh fecal smear with M.Z. N stain oocysts appeared as rose-pink, ovoid, smooth wall. Only C.baileyi can be identified on the basis of morphology alone because it is larger and more ovoid than C.meleagridis and other spp. (Kadir and Yassin, 2002).

Younger chickens are more susceptible to infection with C.bailieyi and will produce more oocysts (Current and Haynes, 1986). as (20) c.ooceysts were reported in 14 of cytological smear of 11 from 20 necropsed birds single infection involving the respiratory or intestinal tract were rare but combined infection involving these two system were frequent (Current *et al.*, 1986) and (Mark A. Goodwin, 1988).

Thin- walled oocysts are auto infective. The thick-walled oosysts are excreted outside the animal (Fayer and Unger, 1986).

In these studies Cryptosporidium spp. Oocyst was reported in 20.4% out 80 cases examined. It has been also demonstrated that Modified Zeihl-Nelseen stain was the best (26.2%) for diction of cryptosporidium oocysts compared to the other two used methods nomely saffranen methylen blue (20%) and Giemsa stain (15%).

The variation in the parentage of the appearance of c.oocyst in cytological smears taken from intestine trachea and bursa of fibricus 18.3%, 8.4% and 1.6% respectfully was dure to tissue tropism of the parasite and also due to organ predlication of the parasite.

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بعض الملاحظات عن مرض الابواغ الخبيئة (Cryptosporidiosis) في الدجاج في مدينة الموصل – العراق

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تم في هذه الدراسة التعرف على طفيلي ذات الابواغ الخبيئة (Cryptosporidiosis) في الدجاج المحلي نوع (Gallus gallus) بواسطة صبغ المسحات المباشر بأستخدام ثلاث صبغات خاصة وهي صبغة زيل نيلسون ، صبغة كيمزا وصبغة سافرانين المثيل الازرق. تم جمع (٨٠) نموذجاً من زرق الدجاج بمختلف الاعمار ومن كلا الجنسين وكان الدجاج يعاني من النحالة واسهال غير دموي ونفوش الريش مع اعراض تنفسية. وقد تبين ان صبغة الزيل نلسون المحورة هي الأحسن في تشخيص الابواغ الخبيئة Oocysts of Croptosporidia ٢٦.٢ % بينما صبغة السافرانين ٢٠ % ، اما صبغة الكيمزا ١٥%. تم تشخيص الطفيلي من مسحات لطخات من الامعاء الدقيقة Scrapings & Imprints Smears والجهاز التنفسي وغدة فابريشيا من الافات المرضية في (٢٠ طيراً مشتبه بأصابتها بالمرض وكانت نسبة الاصابة في الامعاء الدقيقة ١٨.٣ % وفي القصبة الهوائية ٨.٤ % وفي غدة فابريشيا ١.٦ %. ولغرض دراسة التغيرات المرضية النسيجية في بعض الطيور المصابة بالمرض تم جمع ١٠ عينات من الدجاج النافق بد التشريح من الامعاء الدقيقة والقصبة الهوائية وغدة فابريشيا ، وصبغت بصبغة الهيماتوكسلين أيوزين وكانت الافة الرئيسية في الامعاء هي ضمور وتشوه شكل الزغابات المعوية Villi ، فضلاً عن ارتشاح خلايا التهابية (Heterophils Lymphocytes). اما في القصبة الهوائية كانت الافة تنكس مخاطي وانسلاخ الاهداب (Mucinous Degenetion and deciliation). وفي غدة فابريشيا أوضحت الافة بتنكس وفقدان الخلايا اللمفية.