

## 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 (*Gallus gallus*) using three especial stains (Modified Ziehl-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) cereyioered to saffranin methylene blue stains 20% and Giemsa stain 15%. Scrapings and imprints of small intestine trachea and Bursa of fabricous from (20) necropsed 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 propria 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.

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

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### INTRODUCTION

Cryptosporidium is enteric protozoan pathogen, smallest of any coccidian parasite, (phylum Apicomplexa, genus *Cryptosporidium*) that infect human, domestic animals and birds, oocyst size (4-8 X 5-6  $\mu\text{m}$ ) (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.meleagridis* 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 extracytoplasmic 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 cryptosporidia 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 respectivally Oocyst size was 6.4 -3.8µ.

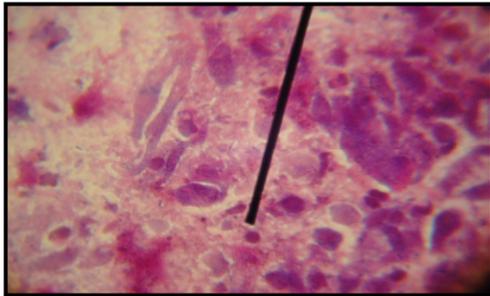
**Table 1:** Comparison of different staining methods in diagnosis of Cryptosporidium oocysts. Using direct method.

| 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               |

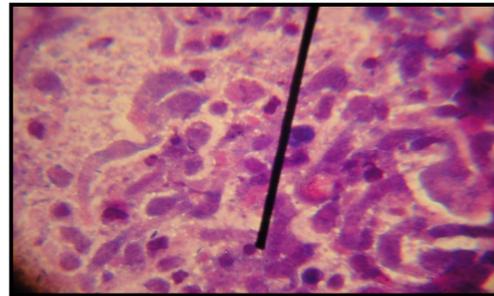
Significant at P<0.05

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

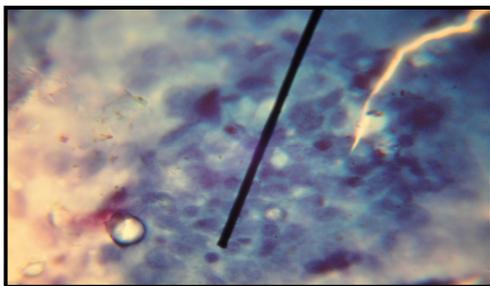
| Stains                             | Small intestine |       | Trachea |      | Bursa of Fabricous |      |
|------------------------------------|-----------------|-------|---------|------|--------------------|------|
|                                    | No +            | %     | No +    | %    | 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 |



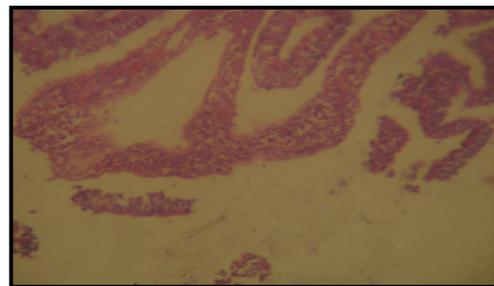
**Fig. 1:** Oocysts of Cryptosporidia from feces stained with Modified Ziehl-Neelsen Stain 1000X



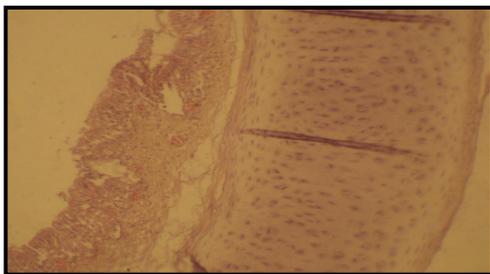
**Fig. 2:** Oocysts of Cryptosporidia from feces stained with Saffranin Methylene Blue stain 1000X



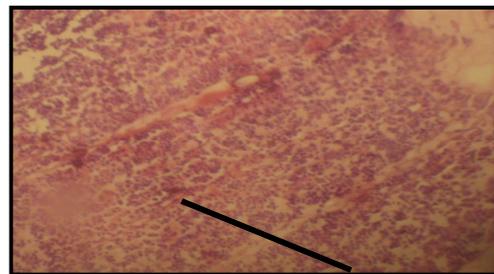
**Fig. 3:** Oocysts of Cryptosporidia from feces stained with Giemsa Stain 1000X



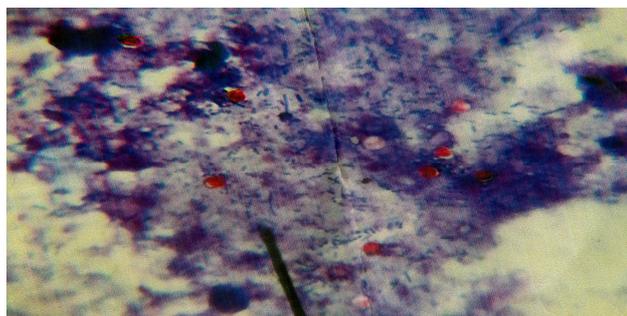
**Fig. 4:** Histopathological section of small intestine of chicken stained with Haematoxyline-Eosin stain 1000X



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



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



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

Putative cryptosporidia. Sp. Oocysts appeared as bright rose-pink smooth spheres ( $5.6\pm 2\mu$ ) on green dark ground by Modified Ziehl-Neelsen stain. Fig (1).

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

Scrapings and impression smears from small intestine, Trachea and Bursa of Fabricus 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 propria was infiltrated by sheets of inflammatory cells (heterophils, macrophages, lymphocytes and plasma cells). Small basophilic spherules ( $5\mu$  diameters *Cryptosporidium* sp.) was embedded within the microvillous border of enterocytes. An obvious signs of chronic enteritis, mucinous degeneration are noticed at intestinal villi with presence of cryptosporidia 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 Fabricus:** Mild changes of Bursal tissue characterized by mild depletion of lymphocyte. accompanied with attachment of the plial 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 cryptosporidia 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. (Arrowood, 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 Ziehl-Neelsen stain method was the best (26.2%) for detection of cryptosporidium Oocysts compared Saffranin-methylene blue (20%) and Giemsa stain (15%). (This is significant at  $P<0.05$ ). The number of samples is too small to judge the sensitivity of these 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.baileyi* and will produce more oocysts (Current and Haynes, 1986). as (20) c.oocysts were reported in 14 of cytological smear of 11 from 20 necropsied birds single infection involving the respiratory or intestinal tract were rare but combined infection involving these two systems were frequent (Current *et al.*, 1986) and (Mark A. Goodwin, 1988).

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

In these studies *Cryptosporidium* spp. Oocyst was reported in 20.4% out of 80 cases examined. It has been also demonstrated that Modified Ziehl-Neelsen stain was the best (26.2%) for detection of cryptosporidium oocysts compared to the other two used methods namely safranin methylene blue (20%) and Giemsa stain (15%).

The variation in the percentage of the appearance of c.oocyst in cytological smears taken from intestine trachea and bursa of Fabricus 18.3%, 8.4% and 1.6% respectively was due to tissue tropism of the parasite and also due to organ predilection of the parasite.

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

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تم في هذه الدراسة التعرف على طفيلي ذات الابواغ الخبيثة (Cryptosporidiosis) في الدجاج المحلي نوع (Gallus gallus) بواسطة صبغ المسحات المباشر بأستخدام ثلاث صبغات خاصة وهي صبغة زيل نيلسون ، صبغة كيمزا وصبغة سافرانين المثل 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 الخبيثة Oocysts of Cryptosporidia 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 مشتبته بأصابتها بالمرض وكانت نسبة الإصابة 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 الناقب بد التشريح من الامعاء الدقيقة والقصبة الهوائية وغدة فابريشيا ، وصبغت بصبغة الهيماتوكسلين أيزون وكان 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.