

FIRST DETECTION OF *CRYPTOSPORIDIUM* SPP. IN BROILER CHICKENS IN SYRIA

Morshed Kassouha

Department of Microbiology, College of Veterinary Medicine,
University of Hama ,Hama, Syria.

(Received 17 December 2013 ,Accepted 29 december 2013)

Key words: *Cryptosporidium* - chicken - Syria .

ABSTRACT

Fifty nine samples of feces were collected from broiler flocks farms located in Hama and Aleppo provinces of Syria, which suffered from diarrhea or respiratory problem or both. For the first time, this study confirmed the infection of the broiler flocks with *Cryptosporidium* in Syria with a rate of 8.4%. The infection has been demonstrated by detecting the *Cryptosporidium* oocysts in the fecal by using direct smear method and Formol-Ether concentration method, then stained by Kinyoun acid fast stain.

The result of tests based on morphology and size of *Cryptosporidium* oocysts showed that the parasite is probably *C. baileyi* which ranged between (6 μ m X 4 μ m).

This study showed a difference in the percentages of infection according to the methods in which *Cryptosporidium* oocysts has been detected, as the Formol-Ether method detected the oocysts in 8.4% of all samples, while the direct smear method detected the oocysts in 6.7%.

INTRODUCTION

Cryptosporidia are small coccidian parasites that were recently recognized as significant pathogens of humans and many other vertebrate species^[1,2,3], The *Cryptosporidium* infection in birds was first described by Tyzzer in 1929^[4], in the cecal epithelium of young chickens. The three valid *Cryptosporidium* species infect birds: *C. baileyi*, *C. meleagridis* and *C. galli* ^[5,6].

Naturally occurring cryptosporidiosis in birds manifests itself in three clinical forms; respiratory disease, enteritis and renal disease, and the parasite has been found in many sites inside the birds including the conjunctiva, nasopharynx, trachea, bronchi, air sacs, small intestine, large intestine, ceca, cloaca, bursa of fabricius, kidneys and urinary tract . Usually only one form of the disease is present in an outbreak^[7] . At present no effective

chemotherapy is available for the treatment of avian cryptosporidiosis^[7,8] .

Although there are intensive studies and huge amount of information about the cryptosporidiosis prevalence in chickens from Europe, united states, American Latin countries and Japan ^[9,10,11,12,13], we found no previous studies on *Cryptosporidium* infections of chickens in Syria.

MATERIALS AND METHODS

3-4 fresh drops have been polled in one container from each of fifty nine suspected broiler flocks and then mixed with 2.5 % K₂Cr₂O₇ and kept at 4°C. The suspected flocks suffered from respiratory or digestive problems or both, and all farms located in two provinces of Syria (Hama and Aleppo) .

Before the examination, the specimen was washed three times with distilled water by centrifugation 1100 x g for 5 min. After washing from potassium dichromate, direct smear (D-S) was made and stained with Kinyoun acid fast stain [14,15] .

Formol-Ether concentration method (F-E) has been administrated on 1ml of washed specimens, and the sediment (figure -1) also stained with Kinyoun acid fast to confirm the result [16].

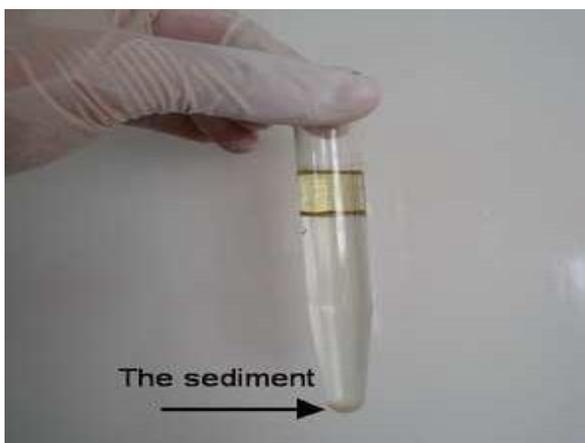


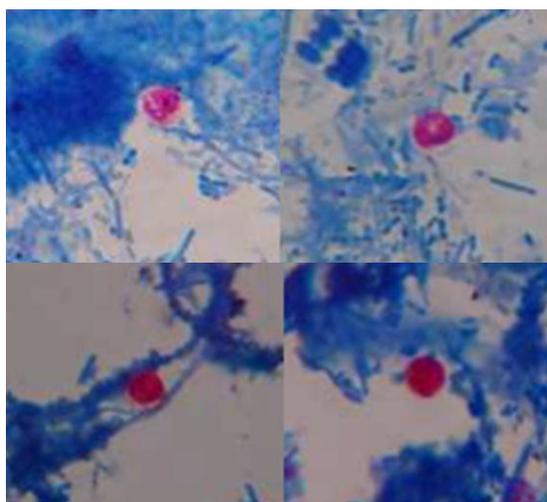
Fig.1: a sample after Formol-Ether method

RESULTS

In this study 5 out of 59 (8.4%) samples were tested positive, one of them was collected from Aleppo province with rate 12.5% (1/8), and the other four positive samples were from Hama province with rate 7.8% (4/51). (Table-1)

In the positive samples under microscope at x 1000 the oocysts look like red or pink spheral bodies against blue background of methylene blue and sometimes the sporozoites can be seen inside it (figure-2).

All oocysts detected ranged in measure 6 μm X 4 μm , and this measurement indicates to *C. baileyi* -like oocyst.



**Fig.2 : *Cryptosporidium* oocysts,(
x2000, kinyoun stain)**

Only four samples were detected using direct smear ((D-S)). Conversely, Formol-Ether concentration method ((F-E)) was able to diagnose all five positive samples. (Table-1)

Table -1 : *Cryptosporidium* detection results according to used methods and source of samples

Source of Sample	D-S method		F-E method		Total	
	N	%	N	%	N	%
Hama (51)	4	7.8	4	7.8	4	7.8
Aleppo (8)	0		1	12.5	1	12.5
Total (59)	4	6.7	5	8.4	5	8.4

DISCUSSION

Just few papers we found about cryptosporidiosis in man and other mammals in Syria , but no one was carried out about *Cryptosporidium* in birds.

In this study we confirmed for the first time the presence of

Cryptosporidium infections In 8.4 % of examined fecal samples from broiler farms in two provinces of Syria.

This rate of infection is low if compared to study from North Carolina [9] that reported 27.3% (9/33), and to another from Greece [12] 24% (17/70), and also low in comparing with two histological studies from northern Georgia [17] and Scotland [11], whereat the parasites were detected in trachea 41% (23/56) and bursa of Fabricius 18.7% ((26/139)), respectively. Otherwise our result was close to that of Georgia [10] which noted the parasite in 6.4% of samples histologically. These differences in infection rates may be due to different environments or different diagnostic methods.

Importance of cryptosporidiosis in chickens has increased recently, especially as it is possibility to be combine with some of the other pathogens and causes greater economic losses, and there are scientific reports pointed to the possibility of participating *cryptosporidium* with both Rio viruses [18] and Marek's Disease Virus [19] resulting in increased mortality and low weight, and it should not be forgotten that *cryptosporidium* infect bursa of Fabricius and cause suppression for immunity, which in turn leads to a lot of bad influences and that might not notice immediately.

In the end, it is necessary to increase the interest and study about of cryptosporidiosis in chickens in Syria and other Arab countries and typing of existing species genetically by PCR-RFLP analysis.

الكشف لأول مرة عن البوغيات الخفية عند دجاج اللحم في سورية

مرشد كاسوحة

فرع الاحياء المجهرية ،كلية الطب البيطري ، جامعة حماه ،حماه ،الجمهورية العربية السورية

الخلاصة

جُمعت تسع وخمسون عينة من مزارع دجاج اللحم في محافظتي حماة وحلب من سورية عانت طيورها من مشاكل تنفسية أو إسهالات أو من الاثنين معاً. أثبتت الدراسة ولأول مرة إصابة قطعان دجاج اللحم بالبوغيات الخفية في سورية بنسبة 8.4%. اذ تم ثبت وجود الإصابة من خلال الكشف عن كيسات بيض البوغيات الخفية في عينات الزرق باستخدام طريقتي اللطخة المباشرة ، وطريقة الفورمول – إيتنر التركيبية ثم تمت الصباغة بطريقة كينون. وبينت نتائج الاختبارات بناءً على الخصائص الشكلية وقياس أبعاد الكيسات البيضية بأن الطفيلي قد يكون من نوع *C. baileyi* حيث كانت ابعادها بين $4 \mu\text{m} \times 6 \mu\text{m}$. وظهرت الدراسة اختلاف نسبة الإصابة بحسب كل طريقة من الطرق التي استخدمت في الكشف عن كيسات بيض البوغيات الخفية، حيث كشفت طريقة اللطخة المباشرة عن احتمال وجود كيسات بيض البوغيات الخفية في 6.7% من العينات المختبرة.

REFERENCES

- 1-Fayer, R., Speer, C.A., Dubey, J.P., 1997. General biology of *Cryptosporidium*. In: Fayer,R. (Ed.), *Cryptosporidium* and *Cryptosporidiosis*. CRC Press, Boca Raton, FL, pp.1–242.
- 2-Nagy, B., 1996. Epidemiologic data on *Cryptosporidium parvum* infection of mammalian domestic animals in Hungary. Magy. Állatorv. Lapja 50, 139–144.
- 3-Tzipori, S., Griffiths, J.K., 1998. Natural history and biology of *Cryptosporidium parvum*. Adv. Parasitol. 40, 6–36.
- 4-Tyzzar, E.E., 1929. Coccidiosis in gallinaceous birds. Am. J. Hyg. 10, 269–383.
- 5-Current, W.L., Upton, S.J., Haynes, T.B., 1986. The life cycle of *Cryptosporidium baileyi* n. s. (Apicomplexa, Cryptosporidiidae) infecting chickens. J. Protozool. 33, 289–296.

- 6-Ryan, U.M., Xiao, L., Read, C., Sulaiman, M., Monis, P., Lal, A.A., Fayer, R., Pavlasek, I., 2003. A redescription of *Cryptosporidium galli* Pavlasek, 1999 (Apicomplexa: Cryptosporidiidae) from birds. J. Parasitol. 89, 809–813.
- 7-Lindsay, D.S., Blagburn, B.L., 1990. Cryptosporidiosis in birds. In: Dubey, J.P., Speer, C.A., Fayer, R. (Eds.), *Cryptosporidiosis in Man and Animals*. CRC Press, Inc., Boca Raton, pp. 133–148.
- 8-Ryan, U.M., Xiao, L., 2008. Avian cryptosporidiosis. In: Fayer, R., Xiao, L. (Eds.), *Cryptosporidium and Cryptosporidiosis*. CRC Press, Taylor and Francis Group, Boca Raton, pp. 395–418.
- 9- Ley, D. H., Levy, M. G., Hunter, L., Corbett, W., and Barnes, H. J., 1988. Cryptosporidia-Positive rates of avian necropsy accessions determined by examination of auramine-O-stained fecal smears, *Avian Dis.* 32: 108-113.
- 10- Goodwin, M. A., Brown, J., 1988. Histologic incidence and distribution of *Cryptosporidium* sp. infection in chickens: 68 cases in 1986. *Avian Dis.* 32(2):365-9.
- 11- Moskovitz, B. L., Stanton, T. L., and Kusmierck, J. J. E., 1988. Spiramycin therapy for Cryptosporidial diarrhea in immunocompromised patients. *J. Antimicrob. Chemother.* 22, 189–191.
- 12- Papadopoulou, C., Xylouri, E., Zisides, N., 1988. Cryptosporidial Infection in broiler chicken in Greece, *Avian Dis.*, 32, 842.
- 13-Sevaa,A., Funada,M., Richtzenhain,L., Guimaraes,M., Souza ,S., Allegretti,L ., Sinhorini,J., Duarte,V. and Soares,r., 2011. Genotyping of *Cryptosporidium* spp. from free-living wild birds from Brazil, *Veterinary Parasitology*, 175 ,27–32.

- 14-Goldman, E., and Green, L. H., 2009. Practical Handbook of Microbiology. Eds. CRC Press, Boca Raton, 2nd ed, London New York, p 853.
- 15- Current, W. L., 1990. Techniques and laboratory maintenance of *Cryptosporidium* in Cryptosporidiosis of Man and Animals, Dubey, J.P., Speer, C.A. and Fayer, R., Eds. CRC Press, Boca Raton, FL, p 31-49.
- 16- Smith, H., 2008. Diagnostics, Fayer, R. Xiao, L., (Eds). *Cryptosporidium* and Cryptosporidiosis CRC Press, Boca Raton, 2nd ed, London New York, p 173-208.
- 17- Goodwin, M. A., Brown, J., 1996. Respiratory Coccidiosis (*Cryptosporidium baileyi*) among Northern Georgia Broilers in OneCompany. Avian Dis, 40:p 572-575.
- 18- Guy, J., Levy, M., Ley, D., Barnes, H.J. and Gerig,T., (1988). Interaction of Reovirus and *Cryptosporidium baileyi* in Experimentally Infected Chickens, Avian Diseases, Vol. 32, No. 3, pp. 381-390.
- 19- Abbassi, H., Coudert, F., Chérel, Y., Dambrine, G., Brugère-Picoux J., and Naciri M.,1999. Renal Cryptosporidiosis (*Cryptosporidium baileyi*) in Specific- Pathogen-Free Chickens Experimentally Coinfected with Marek's Disease Virus, Avian Diseases, Vol. 43, No. 4, pp. 738-744.