Vet. Med. J., Giza. Vol. 46, No. 3. (1998):293-302.

STUDIES ON CRYPTOSPORIDIOSIS OF CHICKENS AT PORT-SAID PROVINCE

H.M. EL-GWADY*, A. A. EL-MELIGY*, M.W. GHATTAS** and S.W. TADROS **

* Faculty of Veterinary Medicine, Suez Canal University
** Animal Health Research Instiute, Port-Said.

Recived :6/11/1997
Accepted :22/12/1997

SUMMARY

Examination of 385 broiler chickens at Port-said Province during the period extending from March 1995 till the end of February 1996 revealed that, 40 (10.38%) were found infected with Cryptosporidun species. The peak of infection occurred during Summer (15.53%), followed by Spring and Autumn (11.66 and 8.77% respectively). The lowest infection rate was found during Winter (4.34%). Morphometrically, the obtained species was identified as Cryptosporidum baileyi. The present study dealt also with the gross pathology and histopathological alterations in the intestine and bursa.

INTRODUCTION

Cryptospordiosis is caused by an apicomplexan protozoon parasite of the genus Cryptospordium, parasitizing the margin of the epithelial cells and appearing to be extracellular (Jordan, 1990). Cryptosporidium species was described many years ago in a wide range of hosts including mammals, birds and man by many authors (Tyzzer, 1907 and 1912); (Slavin, 1955); (Angus, 1983); (Tzipori, 1983) and (Badway and El-Sawy, 1995). Chicken Crypyosporidiosis studies in Egypt are still scarce. So, the present study was done in order to identify the Cryptosporidium species infesting birds in Port-Said Province, Egypt; estimating its seasonal incidence and pathogenicity.

MATERIAL AND METHODS

Intestinal scraping smears of 385 broiler chickens were made on glass slides, air dried, fixed in methyl alcohol, then stained by modified Ziehl-Neelsen stain (Henriksen and Pohlenz, 1981) as well as by Giemsa stain (Pohlenz et al., 1978).

For histopathological studies, tissue specimens from the different parts of the alimentary tract

and bursa were taken immediately after death and tied at their ends. Suitable amount of 10% buffered neutral formalin was injected into the intestinal lumen for rapid fixation of the mucosa. The bursae and intestinal loops were put in 10% neutral formalin.

Dehydration by alcohols, clearing in xylene and embedding in paraffin wax were performed successively. Five microns thickness paraffin sections were obtained by using a rotatory microtome and stained by haematoxylin and eosin (Carleton, 1957).

RESULTS

Out of 385 broiler chickens examined at Port-Said province, 40 (10.38%) were found infected with Cryptosporidium species. The incidence rate was nearly equal at both localities (A&B) where it was 10.04% at A (El-Shark and Port-Foad) as well as 10.79% at B (El-Arab and El-Zohor); Table (1).

Seasonally, Summer showed the highest peak of Cryptosporidium sp. with an incidence of (15.35%) and even among both localities, A (14.28%) and B (17.02%). This was followed by Spring (11.6%) and Autumn (8.77%), while the lowest peak was obtained during Winter (4.76%). The same sequence was obtained among locality A (12.72%, 8.33% and 4% for Spring, Autumn and Winter respectively), while among locality B, Summer was followed by Autumn (11.1%), Spring (10.76%) and finally Winter (5.54%) Table (1).

Monthly, Cryptosporidium sp. infection among broilers was highly represented in August among locality A (17.39%) and locality B (100%), while it was not detected during January in locality A and February as well as September in locality B Table (2).

Examination of the oocysts using the modified Ziehl-Neelsen technique, they appeared ovoid in shape measuring 4.5-6.5 X 4.5.50um in X4-5.50µm size, Fig.(1).

Dealing with the gross pathological lesions in the intestine, 21 out of 40 showed few petechial haemorrhagic foci on the serosal surface of the small intestine. In 19 out of 40, the bursa was slightly enlarged and revealed few petechial haemorrhages on its surface.

Microscopically in the intestine Cryptosporidium species stages were prevalent extracellular and in between the brush border of the villar epithelium Fig.(2). Atrophy and necrosis of the villar epithelium were noticed. The lamina propria revealed inflammatory cellular response with lymphocytes and macrophages. Another cases showed severe pathological response characterized by massive cellular infiltration with lymphocytes, plasma cells, macrophages and few heterophils.

Some birds showed mixed infection of cryptosporidium and Eimeria species. In these cases, Eimeria infection as well as pathological response was mild.

Table (1): Seasonal incidence of *Cryptosporidium* species among chickens examined at different localities at Port - Said Province

Season	Locality	Number of examined chickens	No. of infected	% of infection
Spring	A	55	7	12.72
	B	65	7	10.76
	Total	120	14	11.6
Summer	A	56	8	14.28
	B	47	8	17.02
	Total	103	16	15.35
Autumn	A	48	4	8.33
	B	9	1	11.1
	Total	57	5	8.77
Winter	A	50	2	4
	B	55	3	5.54
	Total	105	5	4.76
Total	A	209	21	10.04
	B	176	19	10.79
	Total	385	40	10.38

Localities A "El- Shark and Port- Foad"
B "El- Arab and El- Zohor"

Table (2): Monthly incidence of *Cryptosporidium* species among chickens examined at different localities at Port - Said Province.

Localities / Month	No. examined		No. infected		% of infection	
	A	B	A	B	A	B
March	20	-	2	-	10	-
April	7	29	1	3	14.28	10.34
May	28	36	4	4	14.28	11.1
June	26	23	3	3	11.53	13.04
July	7	23	1	4	14.28	17.39
August	23	1	4	1	17.39	100
September	25	2	2	0.0	8	0.0
October	12	4	1	1	8.33	25
November	11	3	1	0.0	9.09	0.0
December	14	23	1	1	7.14	3.34
January	12	26	0.0	2	0.0	7.69
February	24	6	1	0.0	4.16	0.0

CamScanner

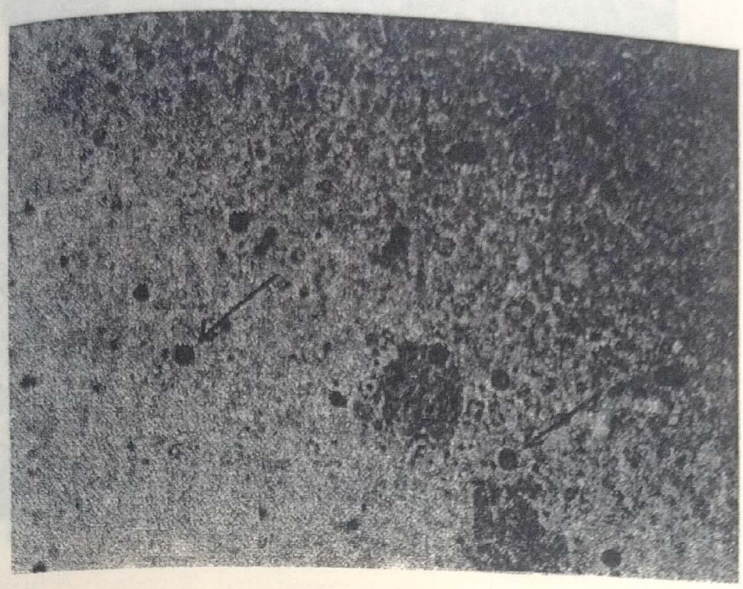

Fig. (1): *Cryplosporidium baileyi* stained with modified Ziehl Neelsen (X 1000).

Fig. (2): Section of intestine of chicken showing *Cryptosporidium baileyi* on the brush border of the villar epithelium (Arrow) H & E X 250.

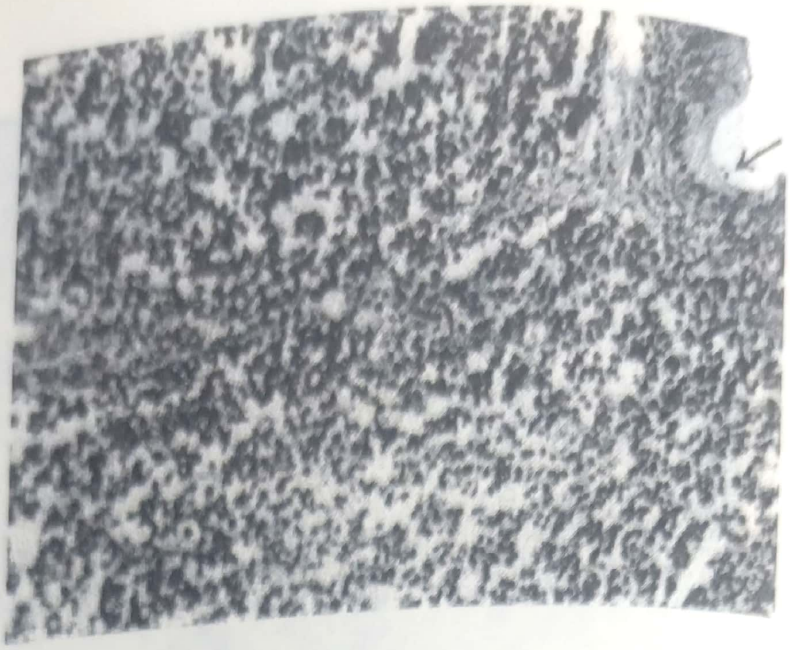

Fig. (3): Section of Bursa of Fabricius of chicken infected with *Cryptosporidial* organisms on the surface of the epithelium (Arrow) H & E X 250.

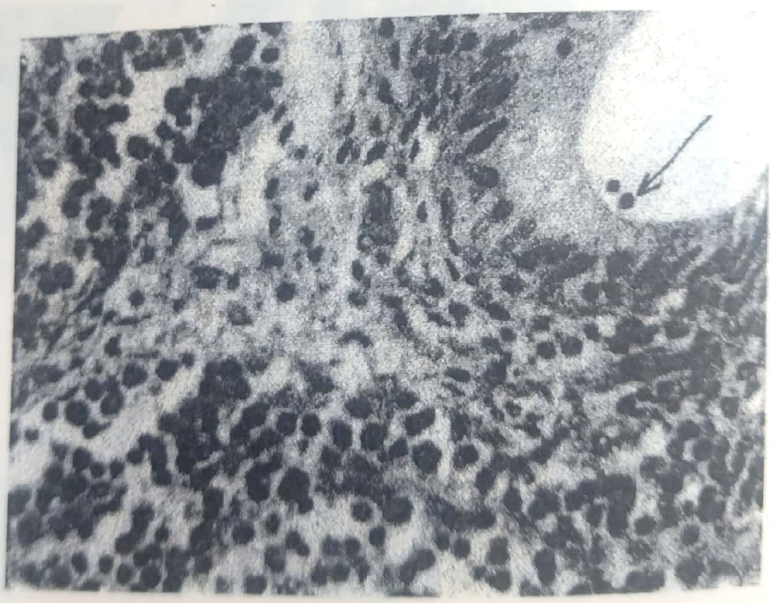

Fig. (4): Higher magnification of Fig. 3 to illustrate the *Cryptosporidium baileyi* on the epithelial surface (Arrow) H & E X 1000.

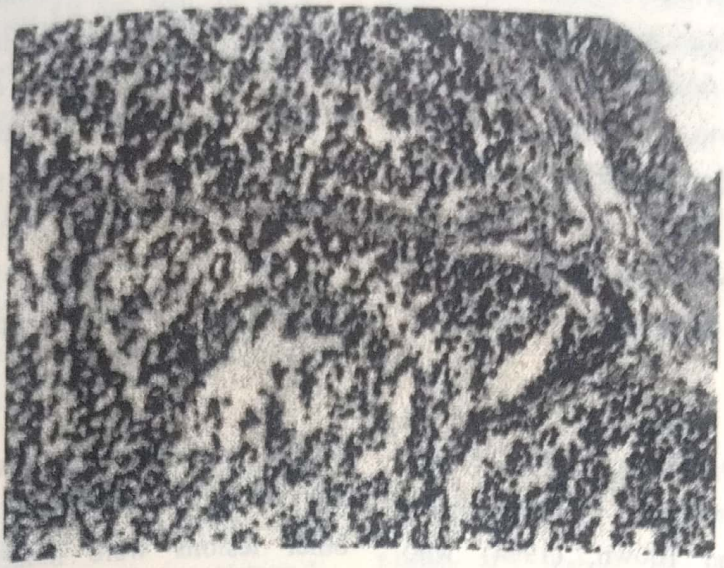

Fig. (5): Section of bursa of Fabricius of chicken infected with *Cryptosporidial* baileyi showing lymphoid depletion (H & E) X 250.

In bursa, the epithelial lining was invaded extracellularly with cryptosporidial developmental stages Fig. (3 and 4), and showed vacuolar degeneration, lymphocytic depletion and necrosis of the lymphoid follicles. Fig. (5).

DISCUSSION

In the present work, Cryptosporidium species was ovoid in shape, measuring 4.5-6.5X4 - 5.50µm in size. These results are in agreement with Georgi et al. (1985); O'Donoghue, (1985); Current et al. (1986); Lindsay et al. (1986); Urquhart et al. (1987); Badway, (1989) and Ahmed, (1992); where they identified their species as Cryptosporidium baileyi similar to that of the present work. On the other hand, Hoerr et al. (1978); Pohenz et al. (1978); Soulsby (1982) and Itakura et al. (1984) mentioned varied measurements for this species.

The incidence of Cryptosporidium species was (10.38%), this is in accordance with Ahmed, 1992 (9.05%) but lower than that recorded by Goodwin and Brown, 1989 (68.8%); Itakura et al., 1984 (36.7%) and (33.3%); Papadopoulou et al., 1988., (24.2%) and Radu and Dan , 1985 (13.63%) and higher than that stated by Goodwin and Brown, 1988 (6.4%). The lower incidence of Cryptosporidiosis in the present study may be attributed, in our opinion, to the strict hygienic measures in poultry houses and using of therapeutic and prophylactic anticoccidial drugs. The peak of infection with Cryptosporidium sp.,

was (15.53%) during Summer followed by Spring, Autumn and Winter (11.66, 8.77 and 4.34% respectively). These results are similar to those mentioned by Ahmed, (1992); while disagreed with Goodwin and Brown , (1988), where the peak was in Spring (8.9%) followed by Autumn, Summer and Winter (6.7,6.3 and 3.5% respectively). These varied seasonal dynamic might be due to the geogrphical locations of varied temperatures.

The lowest incidence during Winter in the present work as well as in the previous studies support Goodwin and Brown, (1994) who mentioned that, cooled weather (freezing) killed Cryptosporidium species oocysts. The use of modified Zeihl-Neelsen and haematoxylin and eosin stain as diagnostic methods appeared effective in identification of Cryptosporidium sp,. as mentioned by Nim et al. (1976); Henriksen and Pohlenz (1981); Angus (1983); Anderson (1984); Urquhart et al. (1987) and Ahmed (1992). The infective pattern with the present species; Cryptosporidium baileyi was extracellular inbetween the brush border of villar epithelium. The accompanied pathological lesions were atrophy and necrosis of the intestinal villar epithelium, in addition to the increased infiltration with lymphocytes and macrophages. These results were in agreement with Jones and Hunt (1983) and Goodwin (1988). In our openion, the increased intensity of inflammatory response may be due to an added secondary infective agent.

In case of bursal infection with Cryptosporidia the parasite was extracellular invador to the bursal epithelium and induced vacuolar degeneration and necrosis of villar epithelium depletion of the lymphoid follicles. These results were partially similar to those mentioned by Fletcher et al. (1975); Tham et al. (1982) and Fernandez et al. (1990), where they added the presence of diffuse hyperplasia of the bursal epithelium and heterophilic infiltration.

In the present study, this type of infection induced mild pathological response while, the other lesions were induced as a result of secondary bacterial infection, in disagreement with Goodwin and Brown (1989) who mentioned that Cryptosporidia infection led to chronic active superficial purulent bursitis with mucosal epithelial hyperplasia.

It was worthy to mention that, in case of mixed infection with Eimeria and Cryptosporidia, the coccidial lesions and intensity of infection were mild due to the previous Cryptosporidian infection.

REFERENCES

Ahmed, H.R.H. (1992): Some studies on Cryptosporidia coccidia of chickens. M.V.Sc. Thesis Fac. Vet. Med. Zagazig. Univ.

Anderson, B.C. (1984): Patterns of shedding of Cryptosporidial oocysts in Idaho calves. J. Am. Vet. Med. Ass., 178 (9): 982-984.

Angus, K.W. (1983): Cryptosporidiosis in man, domestic animals and birds: A review. J.R. Soc. Med., 78: 62-70.

Badawy, B.A. (1989): Fowl Cryptosporidiosis in Egypt. Ph. D. Thesis Parasitology, Fac. Vet. Med. Cairo. Univ.

Badawy, B.A. and El-Sawy, A.M., (1995): Cryptosporidiosis in pigeons. Beni-Suef, Vet. Med. Res. 7 (2): 225-224.

Carleton, H.M. (1957); Histological Technique for Normal and Pathological Tissues and Identification for Parasites. 4th Ed. Exford Univ. Press. New Youk. Toronto.

Current, W.L.; Upton, S.J. and Haynes, T.B. (1986); The life cycle of Cryptosporidium baileyin n.sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. J. proozool., 33 (2): 289-296.

Fernadez, A.; Quezada, M.; Gomez, M.A. and Navarro, J.A. (1990): Cryposporidiosis in chickens from Southern Spain Av. Dis., 34: 224-227.

Fletcher, O.J.; Munnell, F.J. and Page, R.K. (1975): Cryptosporidiosis of the bursa of Fabricius of chickens. Av. Dis., 19 (3) 630-639.

Georgi, J.R.; Theodorides, V.J. and Georgi, M.E. (1985): "Parasitology for Veterinarians" Fourth Ed.W.B. Saunders Company. London.

Goodwin, M.A. (1988):Small intestinal Cryptosporidiosis in a chicken. Av. Dis, 32 (4): 844-848.

Goodwin, M.A. and Brown, J. (1988): Histologic incidence and distribution of Cryptosporidium sp. Infection in chickens. Av. Dis., 32: 365-369.

Goodwin, M.A. and Brown, J. (1989): Light-microscopic lesions associated with naturally occurring bursal Cryptosoporidiosis in chickens Av. Dis., 33: 74-78.

Goodwin, M.A. and Brown, J. (1994); Incidence of respiratory Cryptosporidiosis in Georgia broilers 1987-92., Av. Dis., 338-360.

Henriksen, S.A. and Pohlenz, J.F.L.(1981): Staining of Cryptosporidia by a modified Ziehl-Neelsen technique. Acta. Vet. Scand 22: 594-596.

Hoerr, F.J.; Ranck,, F.M.; Hastings, T.F. (1978): Respiratory Cryptosporidiosis in turkeys. J. Am. Vet. Med. Assoc., 173 (15): 1591-1593.

Itakura, C.; Goryo, M. and Umemura, T. (1984): Crypyosporidial infection in chickens. Av. Pathol., 13: 487-499.

Jones, T.C. and Hunt, R.D. (1983): " Veterinary Pathology" Fifth Ed., pp. 224-245. Bailliere Tindall, London.

Jordan, F.T.W. (1990): "Poultry Diseases" "3"rd Ed. pp. 229. Bailliere Tindall University press, Cambringe, Great Britain.

Lindsay, D.S.; Blgburn,, B.L; Sundermann, C.A.; Hoerr, F.J. and Ernest, J.A. (1986): Experimental Cryptosporidium infections in chickens: oocysts structure and tissue specificity. Am. J.Vet. Res., 47: 876-879.

Nime,F.A.; Burek,J.D.; Page, D.L.; Holscher, M.A. and Yardely, J.H. (1976): Acute enterocolitis in human being infected with protozoa Crypotporidium. Gatro-Enterology 70: 592-598.

O'Donoghue, P.J. (1985): Cryptosporidium infections in man, animals, birds and fish., Aust. Vet. J. 63; 253--258.

Papadopoulou, C.; Xylouri, E.; Zisides, N. (1988): Cryptosporidial infection in broiler chickens in Greece. Av. Dis., 32: 842-843.

Pohlenz, J.; Moon H.W.; Cheville, N.F. and Bemrick, W.J. (1978): Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. J. Am. Vet. Med. Assoc., 172: 452-457.

Radu, S.; Dan, S. (1985): Identification of Cryptosporidium in turkey poults and chickens in Romania. Revista-de-Cresterea-Animaleor., 6: 55-58.

Slavin, D. (1955); Cryptosporidium meleagridis. J. Comp. Pathol., 65: 262-266.

Soulsby, E.J.T. (1982): Helminths, Arthropods and Protozoa of Domesticated Animals "7th Ed." 630-665. The English Book Society and Bailliere, Tindall and Cassel, London.

Tham, V.L.; Kniesberg, S. and Dixon, B.R. (1982); Cryptosporidiosis in quails. Avi. Pathol., 11: 619-626.

Tyzzer, E.E. (1907): A sporozoon found in the peptic glands of the common mouse . Proc. Soci. Exp. Biol & Med., 5: 12-13.

Tyzzer, E.E. (1912): Cryptosporidium parvum (Sp.Nov.) a coccidian found in the small intestine of the common mouse . Arch. Protistenk, 26: 394-412.

Tzipori, S. (1983); Cryptosporidiosis in animals and humans. Microb. Rev., 47: 84-96.

Urquhart, G.M.; Amour J.; Duncan J.L. Dunn A.M. and Jennings F.W. (1987): " Veterinary Parasitology" First Ed. 218-226. Churchill Livingstone Inc., New York.