

Dept. of Poultry
Animal Health Research Institute
Head of Depat. Prof.Dr. Abd-El-Rahman Kafagi

**CRYPTOSPORIDIOSIS IN TURKEY POULTS
DEVELOPMENTAL STAGES AND SENSITIVITY OF
OOCYSTS TO DISINFECTANTS**
(With 3 Tables and 6 Figures)

By

**ENTEISSAR A.A.A., A.A.M. SAHLAB*
and A.I. IBRAHIM***

*: Department of poultry and Fish Disease,
Fac. Vet. Med. Suez Canal Univ.

(Received at 28/1/1997)

طفيل الكريبتوسبورديم في كتاكيت الرومي
اطوار العدوى وحساسية الحويصلات المعديّة للمطهرات

انتصار عرفه ، احمد سحلب ، احمد ابراهيم

تم تشخيص طفيل الكريبتوسبورديم من خلال الفحص الروتيني لكتاكيت الرومي في الجيزة وقد سجلت ٤٦,٧% حالة ايجابية من بين ٥٠ حالة حقلية كان معظمها في الشتاء (٢٢%) بينما كانت نسبة العدوى (٢٤,٧%) موزعة خلال بقية عام (١٩٩٥) هذا وقد تم عزل الطفيل من اللفاتفي و القولون والاعور وحوصلة فابريشي. وصفت اطوار النمو المختلفة بالفحص الميكروسكوبي بعد العدوى الصناعية لكتاكيت الرومي وبالفحص الهستوباثولوجي لوحظ وجود الطفيل على السطح الخارجي المبطن للامعاء المتهتكة مع تضخم في حوصلة فابريشي. تبين ان استعمال الامونيا بتركيز ٥% أدى الى اقوى ابادة للحويصلات في اقصر وقت (٣٠ دقيقة) بالمقارنة بالتركيزات المختلفة للفورمالين والايودوفور.

SUMMARY

In 1995, Out of 450 white turkey poults suffering from emaciation diarrhea and interitis in Giza province, only 46.7% cases were recorded positive to cryptosporidiosis through out the year, 22% in winter and 24.7 was fluctuated through out the other seasons. *Cryptosporidia* was isolated from

ileum, colon, caeca, and bursa of fabricius while it was not observed in the duodenum or trachea. The prepatent and patent as well as the different developmental stages of *Cryptosporidium* parasite in experimentally inoculated turkeys were mentioned. Histopathological studies of affected tissue were done indicating the attachment of organism to epithelial lining of the intestine resulting in vascular degeneration, Edema, proliferation of eosinophils and lymphocytes. The bursa of fabricius, showed depletion of the lymphoid follicles accompanied with attachment of the plical epithelium including the parasitic structure. Using of ammonia 5%, gave the most destructive effect on *Cryptosporidium* oocysts at least time (30 minuts) than formaldehyde or iodophore disinfectants.

Key words: *Turkey Poults - Cryptosporidiosis - Disinfectants.*

INTRODUCTION

Cryptosporidia are coccidial parasites genus *Cryptosporidium*, suborder *Eimeriorina*, (Levine 1982) which develop in an intracellular, extracytoplasmic location (Marcial and Madara 1986) at the apical surface of parasitized host epithelial cells. The life cycle of the *Cryptosporidium* resembles that of other intestinal coccidians, except that there is an autoinfectious cycle and oocysts are shed already sporulated (Tzipori, 1983 and Current, 1985). The duration of oocysts survival in the environment is unknown and the susceptibility of oocysts to the action of disinfectant is difficult (Campbell *et al.*, 1982). Cryptosporidial infection of the intestinal tract and bursa of fabricius have been reported in turkeys and chicken firstly by Slavin (1955) and Naciri *et al.* (1994), as well as in many countries associated with sever enteritis and increase of mortality, but the significance of this infection remains unclear (Lindsay *et al.*, 1986), even it has not been determined whether this parasite was the primary pathogen or played a secondary role with other infectious agents (Glisson *et al.*, 1984). *Cryptosporidium* sp. Have been incriminated as the causative agent in turkeys with respiratory disease and sinusitis (Tarwid *et al.*, 1985, Goodwin *et al.*, 1993,1996 and Hoerr *et al.*, 1987). In other hand, the oral inoculation of turkeys with *Cryptosporidium melagridis* produces infection without clinical signs (Bermudez *et al.*, 1988 and Naciri *et al.*, 1994, while the clinical sings was resulted from the secondary infection (Alex *et al.*, 1988). Literatures about turkey cryptosporidiosis in Egypt are not available so, the purpose of this study is to clarify *Cryptosporidium* infection in turkey poults,

tissue specificity, pathogenicity, prepatent and patent periods, and to study the susceptibility of *Cryptosporidium* oocysts to some disinfectants.

MATERIAL and METHODS

Birds examination for oocyst identification :-

450 turkey poult (commercial flocks) suffering from diarrhea, emaciation and rough feathers from different localities of Giza province were examined for detection of cryptosporidium organism during January through December 1995. The poult were necropsed, gross lesions were observed and microscopical smears were prepared from fecal droppings and from mucosal scrapings of different parts of the intestinal tract, bursa of fabricius and trachea. Stained films with modified Ziehl-Neelsen as special stain for *Cryptosporidia* organism (Henoriksen and Pohlenz 1981) were examined under microscope using oil immersion. Different developmental stages were determined and their diameter were measured and analyzed with the sample mean of Alex *et al.*, (1988) using *t-test*.

Experimental birds

140 turkey poult (40 for the first experiment and 100 for the second one) of one day old of both sexes obtained from commercial hatcheries were housed in wired floored cages. Birds were fed on starter commercial ration without anticoccidial drugs.

Oocysts purification and propagation:

The oocysts collected from the intestinal contents of infected poult were incubated in 1:23 (v/v) peracetic acid for 20 minutes to kill bacterial and viral contamination, (Alex *et al.*, 1988) followed by centrifugation at 1000 xg and the water wash was repeated 5x to remove the peracetic acid. The oocysts were propagated by double passages in turkey poult free from infection. The droppings from the second passage were mixed 1:10 (v:v) solution of sheather's sucrose for levitation of the oocysts (Current *et al.* (1983). The levitated oocysts were washed with distilled water by centrifugation for 5 minutes at 1000 xg. for washing the sheather's sucrose solution. The oocysts were counted using a hemocytometer and a standard suspensions of 10^6 oocysts/ml were prepared for experimental use.

Disinfectants used were:

Formaldehyde 10% (40%, West Molesey Techno cemifal Co.), Ammonia 3% and 5% (25% BDH-LTD. poole-Engeland.), Iodophor 0.3%, 1% and 4% (40%, West Molesey Techno cemifal Co.). One ml of oocysts suspension containing 10^6 oocysts was mixed with 10 ml of each disinfectant

and incubated at 28 °C for 30, 60 minutes and 24 hours then centrifuged and washed twice. Small sample from each mixture was examined microscopically to determine the number of existed oocyst (empty or containing only oocyst residuum or the intact oocyst contains 4 sporozoites). The average percent of oocysts in each incubation time was recorded to study the pathogenesis

First experimental design for studding the prepatent and patent periods, of *Cryptosporidium* oocysts infection:-

Forty turkey poults of one day old of both sexes were housed in isolated wire floored cages. The turkeys were fed on a sterile commercial starter ration without anti coccidial drugs and clean water. At 5 days of age, the poults were individually identified with wing bands and divided into three separate groups.

Birds of group one (10 birds) were served as uninoculated control, While each bird of second group (10 birds) was reared separately and inoculated with 10^6 *Cryptosporidia* oocysts per os by crop gavage. Daily clinical observation, collection of the droppings from each poult and the number of discharged oocysts were recorded upto 30 days. Poults of the third group (20 birds) were given 10^6 *Cryptosporidial* oocysts per os/poult. Two birds were necropsed at day 1,2,3,5,9, 12, 15, 17 and 20 post inoculation (P.I.),to study the developmental endogenous stages. Parts from trachea, Duodenum, Jejunum, ileum, ceacum, and bursa of all turkeys were fixed in 10% neutral buffered formalin and stained with (H&E) for histopathological examination.

Second experimental design for testing the viability of disinfectant treated oocysts:

Sex groups of 15 birds each, and two controls (5 birds each) were used to evaluate the viability of cryptosporium oocysts. the 1st group was inoculated with oocysts previously incubated in formaldehyde 10 % for 30 min,60 min. and 24 hours, the same condition was repeated using Ammonia 3%, 5%, and iodophor 0.33%, 1% and 4% while the seventh control group administered with oocysts was incubated in PBS while the final group was inoculated with PBS. At the end of each time of incubation. four days. post inoculation the droppings from each group were examined daily for detection of cryptosporidium oocyst. One bird from each group was necropsed at day 5, 7, 9, and 15 day post inoculation. Scraping from all intestinal tract was examined microscopically for the presence of endogenous stages of the parasites

RESULTS

Routine examination of 450 cases of turkeys poultts suffering from diarrhea, emaciation and rough feather in Giza province revealed only 210 (46.7%) positive cases for cryptosporidiosis through out the year and 99 cases (22%) in winter and 111 cases (24.7%) were fluctuated through out the other seasons. Cryptosporidial oocysts were detected in fecal droppings of alive birds as well as in scrapings from ileum, colon, ceaca and bursa of fabricius of necropsed birds. The results of experimental study revealed that (in experiment number one) no cryptosporidia were observed neither in fecal droppings nor in the intestinal, bursal, and tracheal scrapings in control groups with no mortality or clinical signs, while inoculated poultts of the second group showed depression and mild diarrhea at day 6 post infection. Cryptosporidial oocysts (Fig. 3a) firstly appeared in droppings at the 5th day post infection and continued for 22 days with a maximum number at 10,17 and 18 days post infection (Fig 1). The gross pathological picture of necropsed birds showed, mucoid secretions and gas bubbles in destinded intestinal tract. Microscopical examination of fecal droppings showed Cryptosporidial oocysts and different developmental stages in scrapings of jejunum, ileum and bursa of fabricius while the trachea was free from the parasites. Histopathological examination revealed that, few *Cryptosporidium* organisms were attached to the epithelial lining of the intestine which also suffered from edema, with infiltration of heterophiles and lymphocytes in the lamina propria (Fig 3b). The bursa of fabricius showed depletion of the lymphoid follicles accompanied with attachment of few round structures to the plical epithelium, on the other hand, The duodenum and trachea had no lesions.

Morphology and dimensions of oocysts and endogenous developmental stages:-

In stained fresh fecal smears with the modified Ziehl-Neelsen's stain, oocysts appeared as acid fast (red pink) ovoid, smooth wall (Fig 3a). The sporozoites were first observed in the mucosal scraping of the jejunum, ileum, large intestine and cloaca at 24 hours P.I. and through 5-19 days P.I. in the bursa. They were long and crescentic in shape (Fig. 4a) the anterior end was more pointed than the other. The nucleus was located near the less pointed end of the sporozoites. The sporozoites exhibit a forward gliding movements. The spherical unnuclated type II meronts (Fig. 5a) were observed at 24 hours P.I. in the mucosal scrapings of the duodenum, jejunum, large intestine and cloaca while the stage was spherical in shape,

contained 8 merozoites and a small spherical residuum attached to the base of the parasitophorous vacuole. Type I merozoites (Fig. 6b) were released from type I meronts. They were observed at 24 hours P.I., in the mucosal scrapings of the duodenum, jejunum, ileum, large intestine and cloaca. These merozoites were nucleated at the middle its long and crescentic in shape with a rounded posterior end tapered to a pointed end. They were motile type exhibiting rapid, forward and snake-like gliding movement. Type II meronts (Fig. 5b) were observed in the mucosal scrapings of the jejunum, ileum, large intestine and cloaca after 48 hours P.I., they were ovoid in shape containing 4 short merozoites and large residuum body. The type II merozoites were released from type II meronts and observed in the mucosal scrapings of the jejunum, ileum, large intestine and cloaca and bursa of fabricius at 72 hours P.I. They were more thicker than type I and the posterior end was broadly rounded while the anterior end was pointed. The nucleus was located near the posterior end. The merozoites exhibited slow, forward gliding movement (Fig. 6c). Meronts type III (Fig. 5c) were observed in the brush border of the jejunum, ileum, large intestine, cloaca and bursa of fabricius at 48 hours P.I.. They were ovoid, containing 4 short merozoites and a large residing body. Type III merozoites (Fig. 6a) were observed in the mucosal scrapings obtained from the jejunum, ileum, large intestine, cloaca and bursa of fabricius at 3 days post inoculation. They were the smallest observed form, often comma-shaped with a broad posterior end that tapered to a pointed anterior end. They exhibit slow forward gliding movement.

Microgamonts:

The microgamonts were seen in the mucosal scrapings of the bursa, cloaca, large intestine, ileum, and jejunum 24 hours P.I.. The microgamonts contain several microgametes surrounding a large homogenous residuum and attached to the base of the parasitophorous vacuole (Fig. 4b). The mature macrogametes (Fig. 4c) were observed for first time in the mucosal scrapings obtained from the jejunum, ileum, cloaca, large intestine, and bursa at 48 hours P.I.. They were spherical forms with a large peripheral nucleus and a prominent refractive granule. The mean measurements of lining endogenous stages of *cryptosporidium melagridis* in mucosal scrapings obtained from experimentally infected turkeys are illustrated in (Table 1).

Distribution of endogenous stages of the organism:

The parasitic distribution (in group 3) was determined by scraping and staining the infected areas of mucosal epithelium of the jejunum which

showed a few to moderate number of *cryptosporidia* on first day upto 20 days (table 2). A few to moderate to sever numbers of cryptosporidia were recorded in the mucosal epithelium of ileum from the second day upto 20th day P.I. while the caecal mucosa of inoculated poult was mild infected with Cryptosporidia at the second day to moderate at 5th day to heavy at 17th day. Distribution of Cryptosporidia in the bursa of fabricius was low at day 5 P.I. and at day 9 was moderate and at 12,15,17,20 days P.I. was heavy. No Cryptosporidia were observed in the duodenum , trachea of all examined poult.

Susceptibility of cryptosporidial oocysts for disinfectants:

Survival of cryptosporidia oocysts after incubation with different disinfectants for 30m, 60m, and 24 hours is presented in (table 3 fig. 2). Results showed that an incubation of oocysts for 30 minutes, only ammonia 5% had 100% destructive effect, while at 60 minutes and 24 hours incubation neither ammonia 5% nor iodophor 4% gave 100% destructive effect. Other disinfectant used resulting in varying degrees of destruction. The viability test in turkey poult indicated that ammonia 5% completely destroyed oocysts infection while iodophor 4% showed 100 % when incubated for 60 minutes and 24 hours. Both formaldehyde 10% and iodophor 0.33% and 1% showed very low effect on oocysts viability.

DISCUSSION

The first report for intestinal cryptosporidiosis in turkey was in 1955 by Slavin and named the parasite *Cryptosporidium melagridis*. The parasite was recorded in a large numbers in the terminal third of the small intestine and was associated with diarrhea and high mortality in 10-14 day old commercial turkeys. Recently, the intestinal cryptosporidiosis has been associated with cases of poult turkey enteritis characterized by diarrhea, reduced weight gain and increased mortality (Goodwin *et al.*, 1988 and Wages 1987). Cryptosporidial infection was widely distributed through turkey poult in Giza province specially in winter causing illness. Specific drug treatment has not yet been recorded. So, attention have been directed toward elimination of the parasite .

Shedding of cryptosporidial oocysts were diagnosed firstly at day 5 post infection and reached the peak at day 10 P.I. then declined at day 12 and gradually increased reaching the maximum number at 17 and 18 days P.I. then sharply decreased and shedding stoped end at 22 days P.I. (Fig. 1). The prepatent and patent period of infection were generally similar to these

reported for experimental cryptosporidiosis in chicken Current *et al.*, 1986, Blagburn *et al.*, 1987 and Ley *et al.*, 1987). The microscopic examination of inoculated poult tissue, indicated the following patency, the infection was being cleared by the host at 22 days P.I. and similar observation have been made by in chicken (Blagburn *et al.*, 1987) and turkey, (Lindsay 1987). Current *et al.*, 1986, reported that clearance of the parasites was due to the acquired immunity of chicken against the reinfection and challenge. Our results indicated that , inoculation with cryptosporidial oocysts by crop gavage resulted in a wide distribution of infected tissue, the indigenous stages of the parasites were found in the sections of Jejunum ,illiem, cecum and bursa of fabricius but not in the trachea as has been reported following the oral inoculation of turkey with *Cryptosporidium baileyi* (Lindisay *et al.*, 1986). Its likely that delivery of oocysts to the oral cavity may result in sporadic infection of the respiratory tract while the inoculation by crop gavage results in the reduction of respiratory tract exposure and infection. Our study revealed that inoculation of turkey poults with 10^6 oocysts by crop gavage developed patent infection with mild clinical signs compared with uninoculated control group. Microscopic observation of infected tissue indicated that, the turkey had low intensity of infection and tissue reaction compared with field cases of cryptosporidiosis-associated poults interitis (Wages, 1987 and Goodwin *et al.*, 1988, 1996). It iss possible that the low intensity of infection found in the present experimental study might be due to low dose of infection, low virulence strain of cryptosporidia. It is also possible that cryptosporidia are opportunistic or secondary pathogens which act in concert with other infectious agents or factors to cause poults interitis. In addition the survival time of the oocysts under condition was unknown. These findings could have important implication in control measures against contamination of poultry houses in the light of our results using of ammonia 5% would be the most appropriate form of decontamination, other disinfectants used showed low destructive effect on oocysts . Ammonia solution to disinfect contaminated houses before new birds are introduced should aid in preventing cryptosporidiosis and offer an advantage than iodophor that give 100% destructive effect after 24 hour incubation.

REFERENCES

- Alex J. Bermudez, Ley A.J.D.H., Levy, M.G., Ficken, M.D., Guy, J.S. and Gerig T.M. (1988): Intestinal and bursal cryptosporidiosis in turkeys following inoculation with *Cryptosporidium* sp. Isolated from commercial poults. Avian Dis., 32: 445-450.
- Bermudez, A.J.D.H. Ley, M.G. Levy, M.D. Ficken, J.S. Guy and T.M. Gerig. (1988): Intestinal and bursal cryptosporidiosis in turkeys following inoculation with *Cryptosporidium* sp. Isolated from commercial poults. Avian Dis. 32: 445-450
- Blagburn, B.L., Lindsay, D.S., Giambronze, G., Sanderman, A. and Hoer, F.J. (1987): Experimental cryptosporidiosis in broiler chicken. Poultry Science, 66: 436-440.
- Compbell, I, Tzipori, S., Hutchison, G. Angus, K.W. (1982): Effect of disinfectants on survival of *Cryptosporidium* oocysts. Vet Record October 30: 414-415.
- Current, W.L. (1985): Cryptosporidiosis J. Am. Vet. Med. Assc. 187: 1334-1338.
- Current, W.L., Reese, N.C., Ernsty, J.R., Bailey-W.S., Heyman, M.B. and Weinstein, W.C. (1983). Human Cryptosporidiosis in immunocomponent and immunodeficient persons. N. Engl. Med., 308 (21): 1252-1227.
- Current, W.L. Upton, S.J. and Haynes, T.B. (1986): The life cycle of *Cryptosporidium bailey* n.sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. J. Protozool. 33: 289-296.
- Glisson, J.R., Brown, T.P., Bnigh, M., Page, R.K., Kleven, S.H. and Davis, R. (1984): Sinusitis in turkeys associated with respiratory cryptosporidiosis. Avian Disease, 28: 783-790.
- Goodwin, M.A. and Brown, J. (1994): Incidence of respiratory cryptosporidiosis in Georgia broilers: 1987-92. Avian Diseases 38: 358-360.
- Goodwin, M.A., Brown, J., Resurreccion, R.S., and Smith, J.A. (1996): Respiratory coccidiosis (*Cryptosporidium baileyi*) among Northern Georgia broilers in one company. Avian Disease, 40: 572-575.
- Goodwin, M.A., Steffens, W.L., Russell, I.D. and Brown, J. (1988): Diarrhea associated with intestinal Cryptosporidiosis in turkeys Avian Diseases 32: 63-67.

- Henoriksen, S.A. and Pohlenz, J.F.L. (1981): Staining of *Cryptosporidium* by Modified Zeil-nielsen technique. Act. Vet. Scand., 22,5, 594-596.
- Hoerr, F.J. Rank, F.M. and Hastings, T.F. (1987): Respiratory cryptosporidiosis in turkeys. J. Am. Vet. Med. Assoc. 173:1591-1593.
- Levine, N.D (1982): Taxonomy and life cycles of coccidia. In: The biology of coccidia. P.L. Long, ed. University Park Press, Baltimore. PP. 1-33.
- Ley, D.H., Guy, J.S., Levy, M.G., Bermudez, A., Barnes, H.J., and Gerig, T.M. (1987): Avian Cryptosporidiosis- an update. Proc. 36th Western Poultry Dis. Conf. Univ. Of Calif., Davis, Calif. PP. 52-54.
- Lindsay, D.S., Blagburn, B.L. and Sundermann, C.A. (1986): Host specificity of *Cryptosporidium* sp. Isolated from chicken. J. parasitol., 72:562-568.
- Lindsay, D.S.B.L., Blagburn and F.J. HOERR (1987): Experimentally induced infections in turkeys with *Cryptosporidium bailey* isolated from chicken. J. Am. Vet. Med. Assoc. 48:104-108
- Marcial, M.A. and Madara, J.L. (1986): *Cryptosporidium*: cellular localization, structural analysis of absorptive cell parasite membrane interactions in guinea pigs and, suggestion of protozoan transport by M cells. Gastroenterology. 90: 583-594.
- Naciri, M., Mancassola, R. Reperant, J.M. and Yvore, P. (1994): Analysis of humeral immune response in chickens after inoculation with *Cryptosporidium bailey* or *Cryptosporidium parvum*. Avian Dis. 38: 832-838.
- Slavin, D.C. (1955): *Cryptosporidium melagridis* (sp. nov.). J. Comp. Pathol. 65: 262-270.
- Tarwid, J.N.R.J. Cawthorn and C. Riddell (1985): Cryptosporidiosis in the respiratory tract of turkeys in saskatchewan. Avian Dis. 29:528-582.
- Tzipori, S (1983): Cryptosporidiosis in animals and humans. Microbiol. Rev. 47: 84-96.
- Wages, D.P. (1987): Cryptosporidiosis and turkey viral hepatitis in turkey poults. J. Am. Med. Assoc.

Table 1: The mean measurements of lining endogenous stages of *Cryptosporidium melagridis* in mucosal scrapings obtained from experimentally infected turkeys

Developmental stages	Mean length	mean width
Sporulated oocysts	5.0-5.5 μm (5.1 \pm 0.02)	4.9-5.4 μm (4.5 \pm 0.025)
Sporozoites	5.7-6.5 μm (5.9 \pm 0.032)	0.9-1.4 μm (1.2 \pm 0.05)
Meronts type I	4.5-5.9 μm (5.5 \pm 0.042)	4.4-5.8 μm (5.4 \pm 0.021)
Merozont type I	4.3-5.5 μm (4.9 \pm 0.041)	0.95-1 μm (0.8 \pm 0.02)
Meronts type II	3.6-5.6 μm (4.6 \pm 0.012)	3.7-5.5 μm (4.6 \pm 0.031)
Merozont type II	4.6-5.4 μm (5 \pm 0.029)	1.3-1.8 μm (1.5 \pm 0.021)
Meronts type III	4.5-5.6 μm (5.1 \pm 0.031)	4.4-4.5 μm (4.3 \pm 0.022)
Merozont type III	3.6-5.1 μm (4.5 \pm 0.04)	1.3-1.5 μm (1.4 \pm 0.023)
Microgamonts	4.5-5.3 μm (5.1 \pm 0.031)	4.5-5.3 μm (5 \pm 0.021)
Macrogamonts	5.2-5.5 μm (5.3 \pm 0.012)	5.1-5.3 μm (5.2 \pm 0.023)
Fertilized macrogamonts	4.9-5.6 μm (5.3 \pm 0.02)	4.8-5.5 μm (5.2 \pm 0.012)

Table 2: The distribution determined by scraped and stained with modified Ziel-Nelsen stain observations of endogenous stages of *cryptosporidia* following incubation of 5 day old turkey poult with 10,000 oocysts by crop gavage.

Days P.I.	<i>Cryptosporidium</i> intensity of infected poults				
	Duodenum	Jejunum	Ileum	Cecum	Bursa
1 day	-	+	-	-	-
2 day	-	+	+	+	-
3 day	-	+	+	+	-
5 day	-	+	++	++	+
7 day	-	+	++	++	++
9 day	-	+	++	++	++
12 day	-	+	++	++	+++
15 day	-	+	++	++	+++
17 day	-	++	+++	+++	+++
20 day	-	++	+++	+++	+++

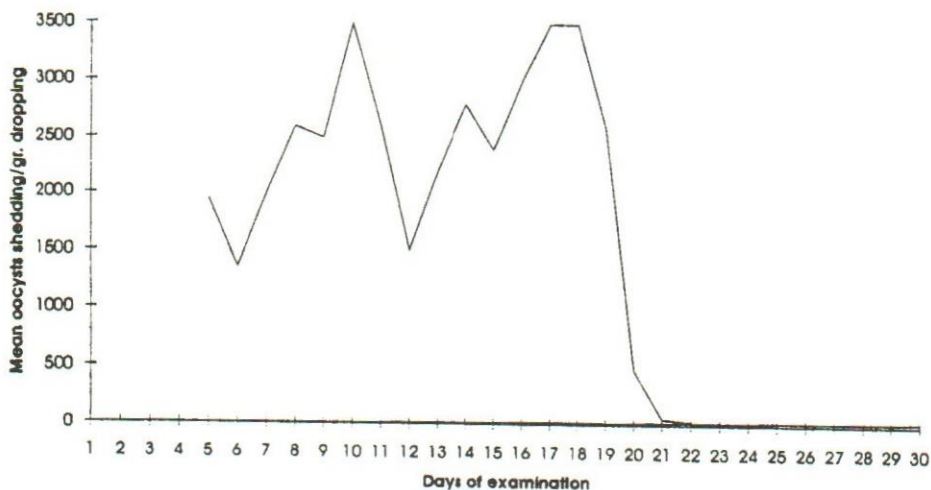
+ = one endogenous stage per microscopic feild (low infection).

++ = two endogenous stages per microscopic feild (moderat infection).

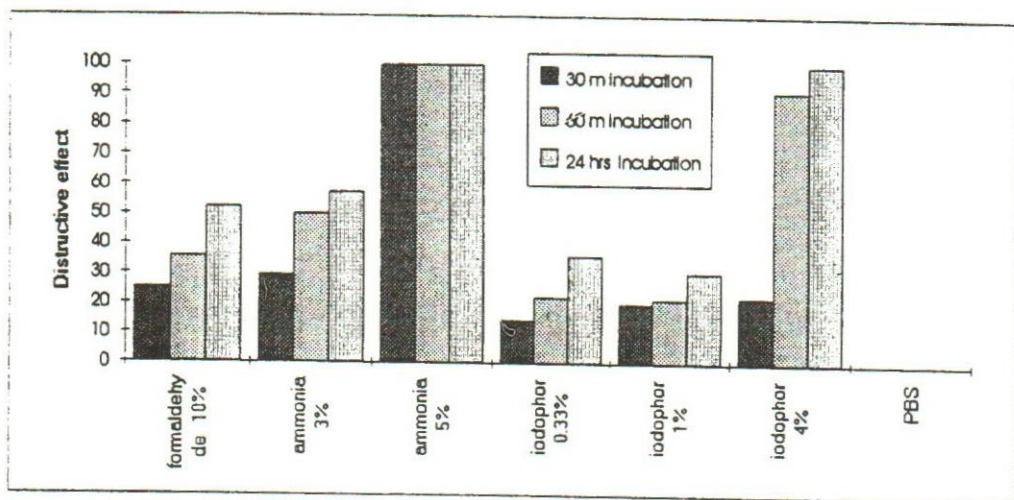
+++ = three or more endogenous stages per microscopic feild (heavy infection)

Table 3: Survival percentage of *cryptosporidia* oocysts after 30, 60 minutes, and 24 hours of incubation with disinfectant

Type of disinfectants	Results after incubation						Results after infection					
	viable oocysts (%)			Damaged oocysts (%)			detection of oocysts in droppings			detection of developmental stages in tissue Scrapings		
	30 m	60 m	24 hrs	30 m	60 m	24 hrs	30 m	60 m	24 hrs	30 m	60 m	24 hr
formaldehyde 10%	75	64.3	47.8	25	35.7	52.2	+	+	+	+	+	+
ammonia 3%	70.6	50	42.9	29.4	50	57.1	+	+	+	+	+	+
ammonia 5%	0	0	0	100	100	100	-	-	-	-	-	-
iodophor 0.33%	85.7	77.8	64	14.3	22.2	36	+	+	+	+	+	+
iodophor 1%	80	78.6	69.6	20	21.4	30.4	+	+	+	+	+	+
iodophor 4%	77.8	9	0	22.2	91	100	+	-	-	+	-	-
PBS	100	100	100	0	0	0	+	+	+	+	+	+
Distilled water	0	0	0	0	0	0	-	-	-	-	-	-

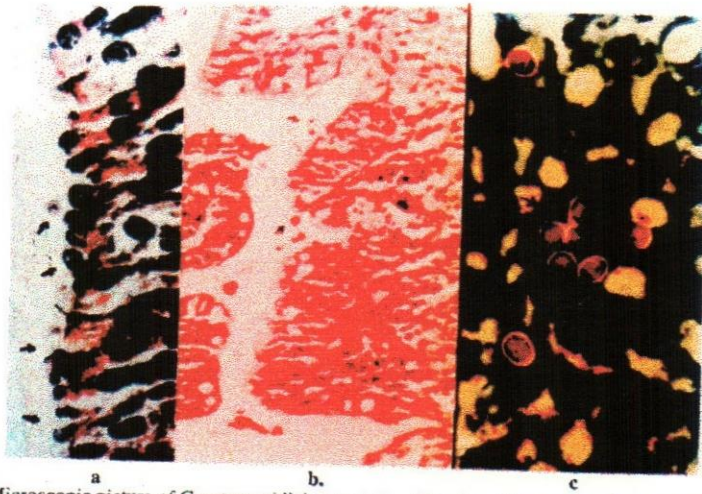


(fig 1) Average daily oocysts output / gr. pooled faeces from experimentally infected turkey poult of group 2.

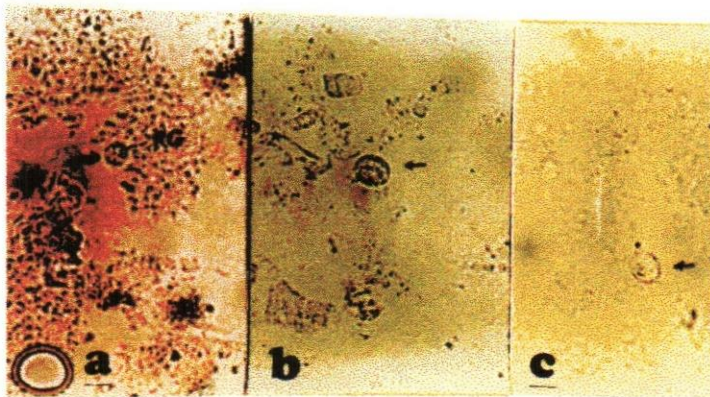


(fig 2) Diagram shows the damaged oocysts after exposure to different disinfectants incubation periods





(Fig 3) Microscopic picture of Cryptosporidial oocysts in **3a**:- fecal droppings after ziehl-neelsen staining x1000 , **3b** :- histological section of intestin (H&E x400), **3c** :- and bursa of fabricius (H&E x400).



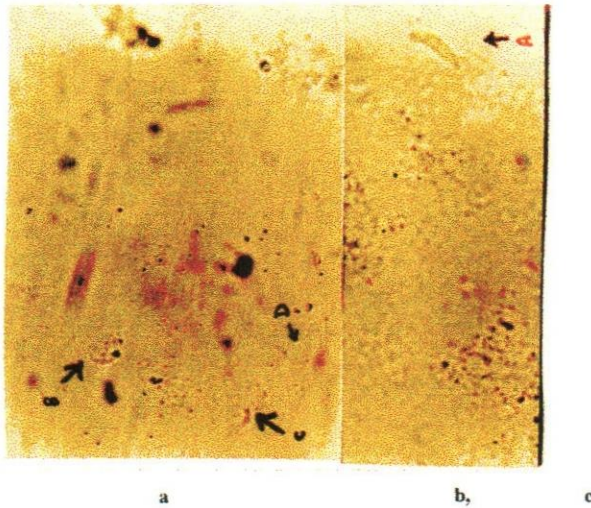
(Fig.4) Microgamonts and Macrogamonts found in intestinal scrappings x1000



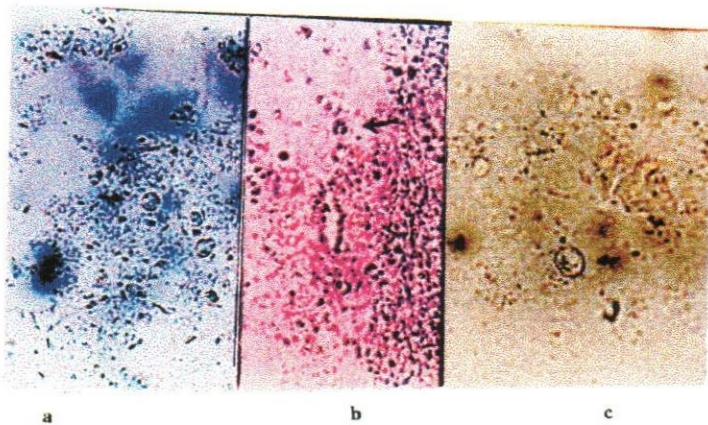
Figure 1. A rectangular area with a dense, multi-colored pattern of blue, yellow, and red dots, resembling a halftone or a textured surface.



Figure 2. A rectangular area with a dense, multi-colored pattern of yellow, red, and blue dots, similar to the one above but with a different color distribution.



(Fig.5) Endogenous developmental meronts type I, II, III found in intestinal scrapings x1000



(fig 6) Endogenous developmental merozoites types :- I, II, III found in intestinal scrapings x1000

THE HISTORY OF THE COUNTY OF MIDDLESEX

