

Competency of commercially available medicaments on treatment of chicken cryptosporidiosis

Reham A. El-Shafei¹, Abdelfattah H. Eladi², Hamed R. Hamed³, and Waleed E. Abou El-Amaiem⁴

¹Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, Egypt

²Department of Poultry Diseases, Faculty of Veterinary Medicine, Mansoura University, Egypt

³Department of Parasitology, Animal Health Research Institute, Mansoura branch, Egypt

⁴Director of Epidemiology Department, Aga District Veterinary Authority, Dakahlia Governorate, Egypt

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ABSTRACT: In the present study the anticryptosporidial efficacy in chickens of two commercially available antibiotics, norfloxacin and spiramycin, in the drinking water alone or in combinations with one commercially available anticoccidial (clopidol) treatment in ration were used. At day 6 of chicken life, 210, male, white Hy-Line chicks were divided into 7 groups (1-7), 30 in each group. Chickens of first group (G1) served as negative control uninfected-untreated group (UUC). Birds of the second group (G2) were inoculated orally with inoculum containing 5×10^5 *Cryptosporidia baileyi* (*C. baileyi*) oocysts/chick and served as infected-untreated positive control group (IUC). All birds of groups 3 to 7 infected with 5×10^5 *C. baileyi* oocysts/ chick and treated with 12mg/kg BW norfloxacin (G3), 1gm/10kg BW spiramycin (G4) from 1st to 5th days post-infection (DPI) and 0.5 gm/kg ration (125ppm) Clopidol (G5) from one day old chick up to one week PI. Last two groups were infected similarly and treated with both of norfloxacin+clopidol (G6) or spiramycin+clopidol (G7) with the same previous dose and course of each treatment. The efficacy of the tested medicaments were evaluated by daily count of *cryptosporidia* oocysts in the feces of chicks, mortality percentage, body weight gain, the degree of histopathological changes of Bursa of Fabricius and lesions score. The results showed that the mortality rate reached 10% in (spiramycin and/or clopidol) treated groups, 6.7% in norfloxacin treated group and 3.3% in norfloxacin+ clopidol treated group while it reached 13.3% in infected-untreated control group. High bursal lesion score was recorded in infected-untreated group followed by infected treated chicken groups regardless the type of medicated drugs. Total oocyst per gram feces of experimentally infected chicks with *C. baileyi* was reduced significantly in all infected treated groups regardless the type of used medicaments when compared with other infected-untreated control chicken group. The recommended dose of norfloxacin and norfloxacin + clopidol decreased the oocyst output of birds by 62% and 63%, respectively. While, other groups showed lower efficiency percentages 43% (spiramycin + clopidol), 40 % (spiramycin) and 35 % (clopidol). Also, non significant differences were observed in body weight gain on day 28 of chicken life between uninfected control and infected groups. Finally, none of these drugs alone can be entirely suggested for the chemotherapy of avian cryptosporidiosis. While, in addition to application of good sanitary measures and disinfection, the present study may conclude that the administration of both norfloxacin in drinking water at 1st to 5th DPI and clopidol in ration one week before and after infection may be helpful in prevention and treatment of *cryptosporidiosis* in chickens.

KEYWORDS: Competency, Cryptosporidiosis, chickens, norfloxacin, spiramycin, clopidol.

INTRODUCTION

Cryptosporidia are related to coccidia, but much smaller (typically oocysts are less than $\frac{1}{4}$ of the size of an *E. acervulina* oocyst). They replicate in the brush border of epithelial cells. They also differ from coccidia in being poorly host specific, although bird strains do not infect mammals very well, and vice versa. *Cryptosporidium baileyi* causes infection of the hindgut and cloacal bursa in chickens, turkeys, and ducks. The oocysts are excreted ready sporulated in the feces and infection occurs by inhalation and ingestion. Unfortunately there is currently no known effective treatment in poultry. If other disease processes are complicating the situation (e.g. coli-septicemia) there may be benefit in medicating for these (Fujino *et al.*, 2002 and McMullin, 2004). Because enrofloxacin and related compounds are widely used drugs in the poultry industry and a preliminary trial indicated some anticryptosporidial efficacy, this drug was included in study presented by Sréter *et al.* (2002) who showed that enrofloxacin when added to drinking water at the recommended level (50 mg/L) from day 1 post-infection (PI) decreased the oocyst output of birds by approximately 50%. However dose increase to 2 times the recommended level did not improve the efficacy or substantially reduce the length of patency. Nevertheless, because some fluoroquinolones seem to be active against apicomplexan parasites, further studies should be undertaken to test the anticryptosporidial activity of fourth-generation fluoroquinolones (Sréter *et al.*, 2002). Many authors reported that oral treatment of spiramycin to cryptosporidiosis in human was effective (Saez-Lioens, 1989; Current and Garcia, 1991 and Ortega, 2007). The sensitivity of field isolates of turkey coccidia from the United States to the anticoccidial drugs amprolium, clopidol, diclazuril, and monensin were investigated by Rathinam and Chapman (2009) who indicated that clopidol and diclazuril were the most effective, followed by monensin and amprolium. Also, efficacy of commonly used anticoccidial drugs in broiler chickens were studied by Hamed and Eladl (2011) who concluded that the administration of toltrazuril in drinking water at 4th to 6th DPI and Clopidol in ration one week, at least, before and after infection was highly effective in treatment and prevention of coccidiosis. They mixed clopidol in ration at a dose of 125ppm equal to 0.5 gm/kg ration at day 14 of chicken life up to one week post challenge with sporulated mixed species of *Eimeria*.

In the present study the anticryptosporidial efficacy of two commercially available antibiotics, norfloxacin and spiramycin, in the drinking water alone or in combinations with one commercially available anticoccidial (clopidol) treatment in ration was evaluated by daily count of *Cryptosporidia* oocysts in the dropping of the experimentally infected chicks. Also, body weight gain, the degree of histopathological changes of bursa of fabricius and lesions score were studied.

MATERIALS AND METHODS

1- Experimental Chicks

A total number of 210, one day old, male, white Hy-Line chicks were supplied by Misr Company for Poultry Production. All birds were reared in cages, kept in strictly isolated room, and were provided with a commercial starter, balanced ration free from any medications, feed and water were provided ad-libitum to all the birds. Lighting was provided for 24 hr throughout the experiment.

2- Preparation of the inoculum and cryptosporidium oocysts

Oocysts of *Cryptosporidium bailey* were isolated from the bursa of Fabricius (BF) taken from large commercial broiler flock in Dakahlia province and identified as described by Hamed (1997). To obtain fresh culture of *Cryptosporidium* oocysts, the isolated oocysts were inoculated orally into twelve of two days old coccidia free chicks, then feces were collected from day 5 to 14 post inoculation as well as mucosal scrapings were obtained from the bursa of Fabricius at the day 14 post inoculation. Oocysts were isolated and purified of from feces and scrapings. Estimation of number of oocysts in the concentrated stock and the infected dose was carried out using the hemocytometer and the light microscope at 40X (Current *et al.*, 1986). Six-day old chicks in this experiment were inoculated orally with inoculum containing 5×10^5 oocysts / chick.

3- Tested medicaments

The following drugs were used as recommended by the manufacturer.

-Norfloxacin: Norfloxacin 20% (Mycomas[®]) produced by Univet, Ireland and was imported by AM trading Company–Egypt. The recommended therapeutic dose is 50mg/Liter drinking water equal to 12mg/kg BW from day-1 up to 5 days post-infection (PI).

-Spiramycin: Spiramycin (Suanovil 50[®]) produced by Coophavet, France and was imported by Tradempex Company–Egypt. The recommended therapeutic dose is 0.4-0.8 gm/Liter drinking water equal to 1gm/10kg BW given from day 1 (PI) up to 5 days PI.

- **Clopidol (Clopidad[®])**: was obtained from Intercova Company-Egypt. The recommended therapeutic dose is 125- 200 ppm, given mixed in ration at a dose equal to 0.5 gm/kg ration from one day old chick up to one week post-infection.

4- Grouping and Experimental design (Table, 1)

A total number of 210, six-day old chicks were divided into 7 groups (1-7), 30 chicks in each group then subdivided into two replicates (15X2). Chickens of first group (G1) served as negative control uninfected-untreated group (UUC). Birds of the second group (G2) were inoculated orally with inoculum containing 5×10^5 *C. baileyi* oocysts/chick and served as infected-untreated positive control group (IUC). All birds in groups 3 to 7 infected with 5×10^5 *C. baileyi* oocysts/ chick. G3 treated with 0.25 ml /liter (12mg/kg BW) norfloxacin . G4 treated with 0.5gm /liter (1gm/10kg BW) spiramycin from day 1 post-infection (DPI) up to 5 DPI ,and G5 treated with 0.5 gm/kg ration clopidol from one day old chick up to one week PI. Chickens in last two groups were infected similarly and treated with both of norfloxacin + clopidol (G6) and spiramycin + clopidol (G7) with the same dose and course of each treatment.

All groups were kept under daily observation for signs, the length of the prepatent and patent periods, body weight, weight gain from day 1 to 21 DPI, mean body weight gain which expressed as relative body weight gain (RBWG), at the beginning of 4th day PI to the 21th DPI total feces were collected daily from each group, weighed and oocysts were counted. Three chicks from each group were sacrificed at 7, 14 and 21 DPI and their bursae were collected for histopathological examination and tissue sections of the bursa of Fabricius were scored.

5- Measuring the efficacy of the tested medicaments

5.1. Clinical symptoms, gross lesions and mortality rates

Signs, gross lesions and number of dead birds were observed according to **Lindsay et al. (1987)**.

5.2. Bursa lesions score

Histologically, the severity of bursa lesion was evaluated according to **Lindsay et al. (1987)**; the score was adopted between 0 and +2. 0=no *Cryptosporidia* present; 1=*Cryptosporidia* lesions present in small or moderate numbers; 2=*Cryptosporidia* lesions present in large numbers.

5.3. Body weight, body weight gain and relative body gain

The body weight and the weight gain were measured from day -1 to 21 DPI to obtain the mean gain which expressed as relative body weight gain (RBWG). Mean body weight values were compared using analysis of variance. Relative body gain was determined according to the method described by **Sréter et al. (2002)**.

5.4. Oocysts count

Beginning day 4 PI (day 4), total feces were collected daily from each group and weighed. The daily output of oocysts in the feces per gram (OPG) of the infected chicks was counted from the 4th to the 21th day post infection by using Mc-Master counting slide (**Current et al., 1986**). Oocysts present in each 24-hr collection of feces were determined by a quantitative method based on the rather slow sedimentation speed of oocysts (**Varga et al., 1995a**). If there were negative results by this method, the fecal samples were examined using Sheather's sugar flotation method (**Current, 1990**).

5.5. Histopathology

It was carried out according to **Tanimura et al. (1995)**. Selected bursae were fixed in 10% neutral formalin, dehydrated in graded alcohol concentrations, cleared with xylene and embedded in paraffin. Embedded tissues were sectioned at 3 μ m and stained with Hematoxyline and Eosin (H&E). Electron Transmission microscopy (E/M) studies were carried out for the detail description of the developmental stages as mentioned by **Lindsay et al. (1986)**.

5.6. Statistical analysis

The obtained data in the present study were statistically analyzed for analysis of variance (ANOVA) and least significant difference (LSD) as described by **Snedecor and Cochran (1981)**.

RESULTS AND DISCUSSION

Control of cryptosporidiosis remains a global challenge in both veterinary and human medicine. Therefore, extensive research has been performed on suitable drugs and disinfectants (**Shahiduzzamana and Arwid, 2012**). The efficacy of the tested medicaments were evaluated by the length of the prepatent and patent periods, daily count of *Cryptosporidia* oocysts

in the feces of the experimentally infected chicks, mortality percentage, body weight gain, the degree of histopathological changes of bursa of Fabricius and lesions score.

1- Effects of the tested medications on Clinical signs, mortality rate and bursal lesion score of experimentally infected chicks with *C. baileyi*.

The results showed that Mortality percentages ranged from 3.3% to 13.3% (1 to 4 dead out of 30 chicks in each infected group) where the mortality rate reached 10% (clopidol) treated group, 10% (spiramycin) treated group, 10% (spiramycin + clopidol) treated group and 6.7% (norfloxacin) treated group, 3.3% (norfloxacin + clopidol) treated group while it reached 13.3% in infected-untreated control group. The negative control (UUC) group was apparently healthy and showed good appetite, good feathering and normal feces. In this group, no mortalities were recorded and showed mortality rate 0%. While, the infected untreated (IUC) group was affected and general symptoms of depression, anorexia, loss of appetite, ruffled feather, nasal discharge and the mortality rate was 13.3% as indicated in Table (2). Similar results were obtained by **Tumova et al. (2002)** who showed that mortality percentages were approximately 10% after infection orally with 5×10^5 oocysts of *C. baileyi*. On the other hand, **Lindsay et al. (1987)** mentioned that deaths not occur; signs or gross lesions were not observed in chicks inoculated orally with oocysts. Concerning to the bursal score lesion our results recorded high bursal lesion score in infected-untreated group (2, 2 and 1) followed by infected treated chicken groups regardless the type of medicated drugs at 7, 14 and 21 DPI, respectively. Lesion scores were recorded lower (1, 1 and 0) in Norfloxacin and Norfloxacin + Clopidol groups than other treated groups (2, 1 and 1) in Clopidol or spiramycin or Spiramycin + Clopidol at 7, 14 and 21 DPI, respectively.

None of the used drugs in this study prevented establishment of *C. baileyi* infections. This lack of efficacy against *C. baileyi* in chickens is similar to that reported for chemoprophylaxis of *C. baileyi* infections in chickens using halofuginone, salinomycin, lasalocid, or monensin (**Lindsay et al., 1987**). clopidol used in the present study was chosen because it was highly effective against *Eimeria* species in chickens (**Hamed and Eladl, 2011**). Although the biochemical mode of action of both Norfloxacin and clopidol is unknown on *C. baileyi* infections, the Clopidol completely controlled the infection with *Eimeria* species on different point of evaluation, mortality percentages, weight gain and oocyst count (**Nicole et al., 1979**). Moreover, clopidol and diclazuril were the most effective anticoccidial drugs against field isolates of turkey coccidia from the United States (**Rathinam and Chapman, 2009**). Because of some fluoroquinolones including norfloxacin seem to be active against apicomplexan parasites, further studies should be undertaken to test the anticryptosporidial activity of fourth-generation fluoroquinolones (**Sréter et al., 2002**).

Enrofloxacin and related compounds are widely used drugs in the poultry industry and a preliminary trial indicated some anticryptosporidial efficacy, this drug was included in study presented by **Sréter et al. (2002)** who revealed that enrofloxacin was added to drinking water at the recommended level (50 mg/L) from day -1 post-infection (PI). The efficacy of enrofloxacin was 52% at the recommended level, although it was not effective against *Cryptosporidium parvum* in neonatal mice (**Blagburn et al., 1991**). **Saez-Lioren (1989)** stated that spiramycin can be used for treatment of intestinal *Cryptosporidiosis* in human. Also, many authors reported that oral treatment of spiramycin to *Cryptosporidiosis* in human was effective (**Current and Garcia, 1991 and Ortega, 2007**).

2-Effects of the tested medications on prepatent, patent periods and total oocysts output in feces of experimentally infected chicks with *C. baileyi*.

The results showed the extension of prepatent period to 5 DPI in groups of spiramycin and/or clopidol, while no extension (4DPI) in groups of norfloxacin and norfloxacin +clopidol when compared with IUC group (4DPI). Also, the results showed the reduction of patent period to 27 DPI in groups of spiramycin and/or clopidol, while the reduction was lower (26DPI) in groups of norfloxacin and norfloxacin +clopidol when compared with IUC (28DPI) group (Table, 3).

Total oocysts per gram (OPG) feces of experimentally infected chicks with *C. baileyi* was reduced significantly in all infected treated groups regardless the type of used medicaments when compared with other infected-untreated control chicken group. The recommended dose of norfloxacin and norfloxacin +clopidol groups decreased the OPG in feces of birds by 62% and 63%, respectively. While, other groups showed lower drug efficiency percentages 43% (spiramycin + clopidol), 40% (spiramycin) and 35% (clopidol) (Table, 3).

These results were confirmed by **Saez-Lioren et al. (1989)** who showed that spiramycin may shorten duration of oocysts excretion and diarrhoea. **Rhee et al. (1998)** found that the pattern of oocysts shedding in chickens inoculated with 5×10^5 oocysts of *C. baileyi* showed that the prepatent period of the protozoa was 2 days and peak oocyst production (10^6 /day/head) occurred between days 8 and 11 PI with a patent period of 30 days while, the oocysts did not show up in the fecal sample of uninfected chickens during this period. Also, **Hao et al. (2008)** showed that the total oocysts excretion levels of *C. baileyi* were detected consistently in feces from the infected chicks but were not observed in the control group. On a similar

ground, **Sréter et al. (1999)** showed that the recommended dose of toltrazuril decreased the oocyst output of birds by 52.1%. The recommended anti-*Eimeria* dose of diclazuril did not reduce significantly the *Cryptosporidium* oocysts output of birds (14.6%). Similarly, **Sréter et al. (2002)** calculated the extension of prepatent period on the basis of the prepatent period of the IUC birds (5 days) and found that extension of prepatent period 0% in enrofloxacin and 10% or 30% in paromomycin according to dose. But the reduction of patent period calculated on the basis of the patency of the IUC birds (13 or 14 days on 2 experiments) and found that the reduction of patent period 8 and 15% in enrofloxacin and 12 and 23% in paromomycin. They also calculated the efficacy percentages on the basis of the total oocysts output of the IUC birds ($29.3-33.8 \times 10^6$) and found that the recommended antibacterial dose of enrofloxacin decreased the oocyst output of birds by approximately 50%. They added that dose increase did not improve the efficacy or substantially reduce the length of patency. At the recommended levels, paromomycin decreased output of oocyst by 67-82%, showing the highest efficacy of all drugs tested against avian cryptosporidiosis thus far. Moreover, the patent period was shortened by 12-23% (**Sréter et al., 2002**). Moreover, they showed that the longer prepatent and shorter patent periods in paromomycin-treated group when compared to control group. On the other hand, several commonly used disinfectants were tested for their ability to kill *C. baileyi* or *C. parvum* oocysts, but only a few of them were effective at concentrations recommended by the manufacturers (**O'Donoghue, 1995**). Potentiation of ionophorous anticoccidials with Duokvin (antioxidant) against *C. baileyi* in chickens was studied by **Varga et al. (1995b)** who showed that none of the treatments represented a major progress in the total oocyst output in the different groups used for the comparison of the anticryptosporidial efficacy. These relative efficacies confirm poor salinomycin, lasalocid, and monensin activity against *C. baileyi* (**Lindsay et al., 1987**). Therefore, **Varga et al. (1995b)** concluded that none of the polyether ionophores or their combinations with duokvin (antioxidant) can be recommended for the chemotherapy or control of avian cryptosporidiosis. This study also confirmed the unusual incompetency of *Cryptosporidium* spp. to various drugs (**Fayer, 1993**).

3-Effects of medications on average body weight (gm), body weight gain (gm), RBWG, bursa weight and BBI of experimentally infected chicks with *C. baileyi*.

Average bodyweight, Body weight gain (gm), RBWG, Bursa weight, BBI at 28 day old of experimentally infected chicks with *C. baileyi* were summarized in Table (4). There were no significant differences observed in final live weight on day 28 of chicken life between uninfected control and infected groups. Similar results were obtained by **Tumova et al. (2002)** who studied the effects of experimental cryptosporidiosis, broiler chickens were infected *per os* with 5×10^5 oocysts of *C. baileyi*. They showed that although the clinical signs of infection were apparent, neither final live weight nor mortality was significantly influenced in chickens infected with a single *Cryptosporidium* species.

In this study, there was no significant difference in the body weight gain between UUC and IUC and also no significant differences were observed in Body weight gain on day 28 of chicken life between uninfected control and infected-treated groups. Based on the past literatures the *C. baileyi* did not cause a weight gain reduction in orally inoculated birds, so no weight gain reduction in IUC group compared to UUC group (**Varga et al., 1995b; Hornok et al., 1996; and Sréter et al., 1996**). Similarly, this is in line with other observations confirmed a lack of weight gain reduction in chickens orally infected with *C. baileyi* (**Lindsay and Blagburn, 1990**). **Sréter et al. (1999)** found that the efficacy of toltrazuril was 52.1% at the recommended level, which could be moderately increased using 5 or 10 times the recommended dose. However, these doses resulted in significant weight gain reduction. Similar results were also obtained by **Sréter et al. (2002)** who found that the body weight gain of paromomycin-treated chickens was almost identical with that of uninfected, untreated control birds irrespective of dosage, indicating the lack of toxicity of the drug for chickens. That drug exhibited the highest efficacy of all drugs tested against avian cryptosporidiosis so far.

Results of bursa weight and bursa body weight index (BBI) on day 28 of chicken life showed that chicks infected with *C. baileyi* had lower bursa weights, leading to significantly lower BBI in IUC (0.14 ± 0.07), than UUC (0.35 ± 0.06) and infected treated groups (Table, 4). Similarly, **Varga et al. (1995b)** found that the co-administration of ionophores with duokvin resulted in increased efficacies but showed significant growth depression and bursa weight reduction in all groups. They also stated that there was a close negative correlation between the efficacy of drugs and the relative weight of the bursa of Fabricius of the experimental groups. Thus, the reduced oocysts production might be explained by the diminished area of the site of *Cryptosporidium* multiplication, which is known to be the bursa of Fabricius.

4- Histopathological findings

Plate (1) showed histopathological changes. Bursa of Fabricius smear stained with Modified Ziehl-Neelsen stain from treated group with norfloxacin+clopidol at 14 DPI showed little stained *Cryptosporidial* oocysts and other obvious oocysts remained unstained. The stained oocysts appear ovoid or rounded acid fast bodies with internal granules against green background (**Fig. A**). Infected untreated group (IUC) at 14 DPI showed more *Cryptosporidial* oocysts stained with Modified Ziehl-Neelsen stain (**Fig. B**), if it compared with that in **Fig A**. Sections for transmission E/M were applied to get more and fine

details about the effect of treatment used in this experiment. Exactly the picture of the examined sections showed different size of vacuoles and little endogenous stages on the brush border of the epithelium in which may be revealed to degeneration of these stages leaving free vacuoles or was hidden. Parallel finding was showed the stained bursal smear in treated group (**Fig. A**). While more oocysts do not take the stain rather than that was showed in **Fig. B**. The previous above mentioned observations may be a result of success to the combination of drug used in this treated group. Transmission E/M of spiramycin treated group at 14 DPI contained many vacuoles and little undeveloped endogenous stages on the epithelial brush border (**Fig. T1**). Transmission E/M of Clopidol treated group at 14 DPI showing little stages like microgamete, zygote, free sporozoites and vacuoles (**Fig. T2**) Infected untreated group (IUC) at 14 DPI revealed endogenous developmental stages stained with toluidine blue (**Fig. U1**). Also, infected untreated group (IUC) at 14 DPI with E/M showed freshly multiple endogenous developmental stages on the brush border (**Fig. U2**).

Rhee et al. (1997) found that the most parasites occurred between days 4 and 16 PI. No *Cryptosporidia* were observed from the 28th day PI downward. They also showed that the prepatent period was 2 days and peak oocyst production occurred between 7 and 10 days. Infiltration of heterophils was first observed on day 4 PI. It increased to high numbers between days 12 and 16 PI and disappeared from the 28th day PI downward. On day 24 PI, a great deal of characteristic feature of *Cryptosporidium*-infected BF was visualized. The hyperplastic alterations were pronounced from the 44th to 68th days PI. It is interesting that hyperplasia of the epithelial cells and thickening of the epithelium were not repaired normally until day 100 PI. **Banai et al. (2002)** showed that *C. baileyi* is a primary pathogen and can induce clinical respiratory disease and other pathogens can enhance the severity of *C. baileyi*-induced disease in chickens. Because of immunosuppressive agents, the course of infection may last more, most cryptosporidia infections in broiler flocks occurs in the last weeks of their life. Moreover, **Banai et al. (2002)** showed that examination of fresh mucosal scrapings revealed endogenous stages of *Cryptosporidium* sp. in apical portion of the epithelium of the BF and cloaca. Oocysts concentrated by Sheater's sugar flotation and viewed with light microscopy, appeared as translucent ovoid to spherical bodies containing one to four dark granules. In acid fast staining, oocysts showed red staining against a green or blue background. Histopathological sections of the BF revealed *Cryptosporidia* of various sizes adherent to the surface of lining epithelial cells. Based on the parasite localization, hosts, size and shape of the oocysts it was concluded that the parasite appeared to be *C. baileyi*. The average size of oocysts measured by micrometry was 4.5-6.5µm. *C. baileyi* differs in site of development from *C. meleagridis*.

CONCLUSION

None of norfloxacin, spiramycin or clopidol alone can be entirely suggested for the chemotherapy or chemoprophylaxis of avian cryptosporidiosis. While, in addition to application of good sanitary measures and disinfection, it may be concluded that the administration of both norfloxacin in drinking water at 1st to 5th DPI and clopidol in ration one week before and after infection may be helpful in prevention and treatment of cryptosporidiosis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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APPENDIX

Table 1. Grouping and Experimental design

Group		Number of birds	Infection orally with 5×10^5 <i>C. baileyi</i> oocysts/chick (6days)	Treatment (dose & course)
G1	Uninfected-untreated negative control (UUC)	30 (15X2 replicates)	-	-
G2	Infected-untreated positive control (IUC)	30 (15X2 replicates)	+	-
G3	Norfloxacin	30 (15X2 replicates)	+	0.25 ml /Liter equal to 12mg/kg BW, from 1-5DPI
G4	Spiramycin	30 (15X2 replicates)	+	0.5gm /Liter equal to 1gm/10kg BW, from 1-5DPI
G5	Clopidol	30 (15X2 replicates)	+	0.5 gm/kg ration equal to 125ppm, from one day old chick up to one week PI
G6	Norfloxacin+Clopidol	30 (15X2 replicates)	+	Similar dose and course for each one
G7	Spiramycin+Clopidol	30 (15X2 replicates)	+	Similar dose and course for each one

Table 2. Effect of Norfloxacin, Spiramycin, Clopidol, Norfloxacin+Clopidol and Spiramycin+Clopidol on mortality percentage and bursal lesion score in experimentally infected chicks with *C. baileyi*.

Group		Mortality percentage*		Lesion score (n=3)		
		Dead/total	Mortality percentage	7 DPI	14 DPI	21 DPI
G1	Negative control (UUC)	0/30	0%	0	0	0
G2	Positive control (IUC)	4/30	13.3%	2	2	1
G3	Norfloxacin	2/30	6.7%	1	1	0
G4	spiramycin	3/30	10%	2	1	1
G5	Clopidol	3/30	10%	2	1	1
G6	Norfloxacin+Clopidol	1/30	3.3%	1	1	0
G7	Spiramycin+Clopidol	3/30	10%	2	1	1

*Mortality percentages ranged from 3.3% to 13.3% (1 to 4 dead out of 30 chicks in each infected group). 0=no cryptosporidia present; 1=cryptosporidia present in small number or moderate numbers; 2=cryptosporidia present in large numbers. UUC = Uninfected-untreated control; IUC=Infected-untreated control; DPI= Day Post Infection

Table 3. Effect of Norfloxacin, Spiramycin, Clopidol, Norfloxacin+Clopidol and Spiramycin+Clopidol on prepatent, patent periods and total oocyst output in feces of experimentally infected chicks with *C. baileyi*. (Mean \pm SE) $\times 10^6$, n=5.

Group		Prepatent Period (DPI)	Patent Period (DPI)	Total oocyst output and drug efficiency%		
				oocyst per gram feces(OPG)	Percentages to IUC	Drug Efficiency%
G1	Negative control (UUC)	0.0	0.0	0.0 ^d	0.0	0.0
G2	Positive control (IUC)	4	28	124 \pm 1.6 ^a	100	0.0
G3	norfloxacin	4	26	47.1 \pm 1.1 ^c	38	62
G4	spiramycin	5	27	74.4 \pm 2.1 ^b	60	40
G5	Clopidol	5	27	80.6 \pm 1.8 ^b	65	35
G6	Norfloxacin+Clopidol	4	26	45.9 \pm 0.9 ^c	37	63
G7	Spiramycin+Clopidol	5	27	70.7 \pm 1.5 ^b	57	43

UUC = Uninfected-untreated control; IUC=Infected-untreated control

The different letters at the same column mean that there was a significant change at $p < 0.05$.

Table 4. Effect of norfloxacin, spiramycin, clopidol, norfloxacin+clopidol and spiramycin+clopidol on average body weight (gm), Body weight gain (gm), RBWG, bursa weight, BBI at 28 day old of experimentally infected chicks with *C. baileyi*. (Mean \pm SE) n=10

Group		Average body weight at 7days	Average body weight at 28days	Body weight gain (gm)	RBWG (%)	Bursa weight* at 28days (n=3)	BBI*
G1	Negative control (UUC)	80 \pm 2.1	231 \pm 4.1	151 \pm 0.6	100	0.82 \pm 0.13 ^a	0.35 \pm 0.06 ^a
G2	Positive control (IUC)	79 \pm 1.4	228 \pm 3.6	149 \pm 0.8	98.7	0.31 \pm 0.11 ^e	0.14 \pm 0.07 ^d
G3	Norfloxacin	77 \pm 1.3	227 \pm 2.7	150 \pm 0.2	99.3	0.62 \pm 0.10 ^b	0.27 \pm 0.01 ^b
G4	spiramycin	76 \pm 2.4	226 \pm 3.4	150 \pm 0.3	99.3	0.51 \pm 0.19 ^c	0.23 \pm 0.02 ^{bc}
G5	Clopidol	76 \pm 1.9	225 \pm 2.9	149 \pm 0.4	98.7	0.45 \pm 0.16 ^d	0.20 \pm 0.05 ^c
G6	Norfloxacin+Clopidol	77 \pm 2.9	227 \pm 3.1	150 \pm 0.5	99.3	0.56 \pm 0.18 ^b	0.27 \pm 0.09 ^b
G7	Spiramycin+Clopidol	77 \pm 3.1	225 \pm 3.9	148 \pm 0.1	98.0	0.54 \pm 0.11 ^b	0.24 \pm 0.08 ^{bc}

RBWG= Relative body weight gain expressed as a percentage of the uninfected, untreated control. RBWG =body weight gain/UUC body weight gain $\times 100$. BBI= Bursa Body weight index=Bursa weight/Body weight $\times 100$ UUC = Uninfected-untreated control; IUC=Infected-untreated control; the different letters at the same column mean that there was a significant change at $p < 0.05$.

Plate (I) showed the histopathological lesions as follows:

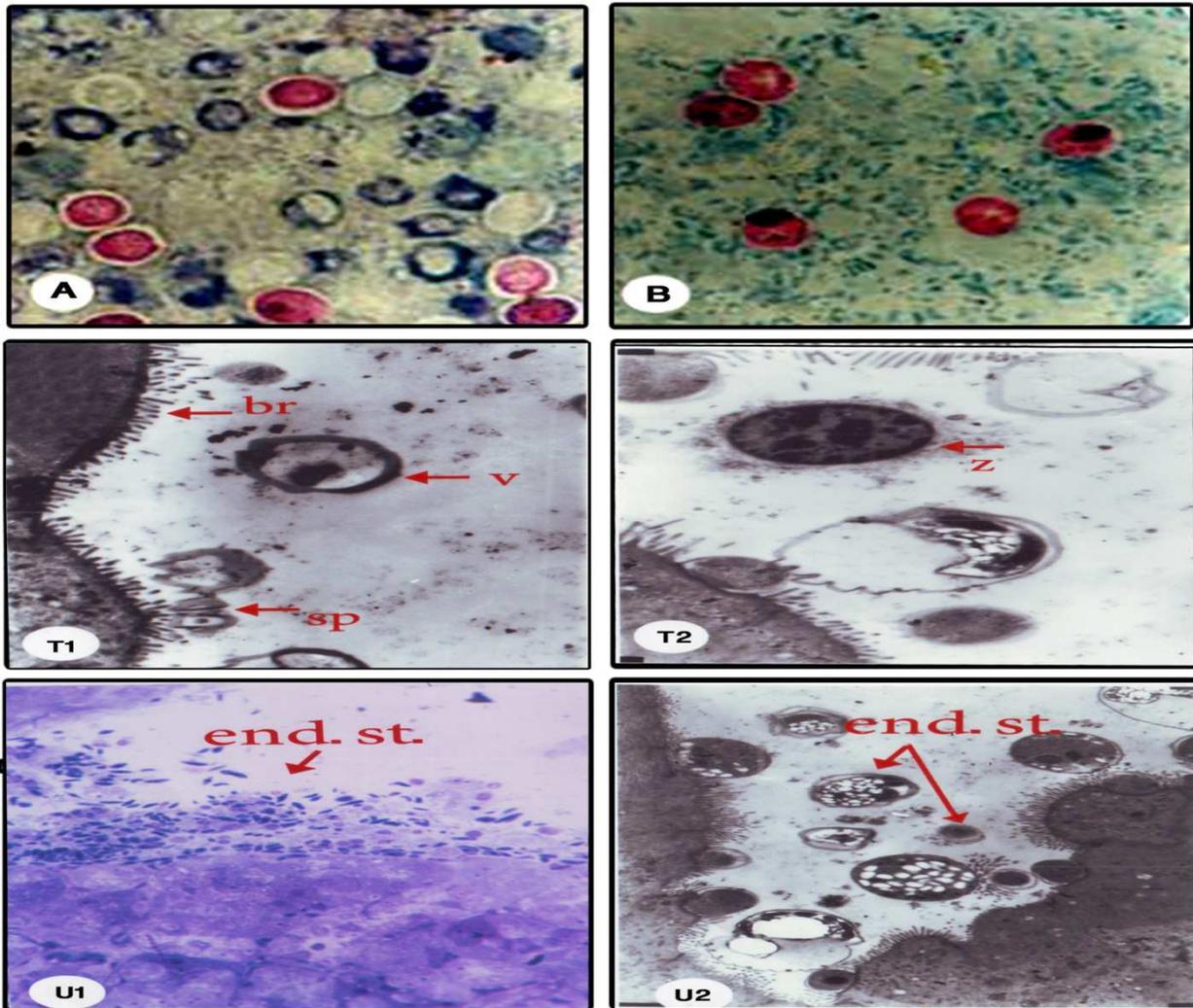


Fig (A): Bursal smear stained with Modified Ziehl-Neelsen stain from treated group with norfloxacin+clopidol at 14 DPI: showed little stained *Cryptosporidial* oocysts and other obvious oocysts remained unstained. The stained oocysts appear ovoid or rounded acid fast bodies with internal granules against green background X1000.

Fig (B): Infected Untreated group (IUC) at 14 DPI showed more *Cryptosporidial* oocysts stained with Modified Ziehl-Neelsen stain, if it compared with that in Fig A. X1000

Fig T1: Transmission E/M of spiramycin treated group at 14 DPI showing contained many vacuoles and little undeveloped endogenous stages on the epithelial brush border.

Fig T2: Transmission E/M of Clopidol treated group at 14 DPI showing stages little stages like microgamete, zygote, free sporozoites and vacuoles.

Fig U1: Infected Untreated group (IUC) at 14 DPI revealed endogenous developmental stages stained with toluidine blue. X1000

Fig U2: Infected Untreated group (IUC) at 14 DPI with E/M showed freshly multiple endogenous developmental stages on the brush border.

br= brush border v= vacuole sp= sporozoites Z= zygote end.st= endogenous stages