Transactions of the Illinois Academy of Science
(1986), Volume 79, 3 and 4, pp. 149-156

DRUG EFFICACY AGAINST COCCIDIAN PARASITES IN GAME-FARM REARED PHEASANTS *(PHASIANUS COLCHICUS)* FROM ILLINOIS

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ABSTRACT

Litter samples were obtained from an Illinois ring-necked pheasant propagation farm to determine (1) the species of coccidian parasites present in the birds and (2) the coccidiocidal or coccidiostatic action of sulfaquinoxaline, amprolium and furazolidone against these parasites. Four coccidial species were found: *Eimeria duodenalis* represented 57.5% of the oocysts recovered, *E. tetartooimia* represented 24.9%, while *E. phasiani* and *E. pacifica* showed about equal numbers of oocysts totalling 17.6%.

All three drugs were effective in reducing oocyst production compared to non-medicated controls. Furazolidone showed no coccidiostatic effect against any of the coccidial species and its coccidiocidal action was not sufficient to prevent mortality (42.8% vs. 71.4% for non-medicated group). Amprolium prevented mortality but was relatively ineffective against *E. tetartooimia* and *E. duodenalis* and was partially coccidiostatic against *E. phasiani* and *E. pacifica*. Sulfaquinoxaline was the most effective drug in controlling the coccidial parasites. It had complete coccidiostatic activity against all four coccidial species and a slightly stronger coccidiocidal action against *E. duodenalis* and *E. tetartooimia* than *E. phasiani* and *E. pacifica*.

INTRODUCTION

Game farms produce millions of ring-necked pheasants annually for food and hunting. The management practices of these game farms are often similar to those used in the poultry industry. Coccidiosis and other parasitic diseases which are familiar to the poultryman frequently occur and cause weight loss, poor feed conversion and mortality in pheasant flocks as well as in poultry.

Eight coccidial species have been described from pheasants (Todd and Hammond, 1971; Tyzzer, 1929; Wacha, 1973). Two species, *Eimeria langerani* (Yakimoff and Matschoulsky, 1937) and *E. colchici* (Norton, 1967a) were reported from Europe. *Eimeria phasiani* (Tyzzer, 1929) and *E. duodenalis* (Norton, 1967b) have been reported in Europe and the U.S. (Fisher and Wacha, 1976; Haase, 1939; Ormsbee, 1939; Trigg, 1967a; Wacha, 1973) and four species were reported only in the U.S.: *E. megalostomata* (Ormsbee, 1939), *E. pacifica* (Ormsbee, 1939), *E. dispersa* (Tyzzer, 1929) and *E. tetartooimia* (Wacha, 1973; Fisher and Wacha, 1976).

Anticoccidial drugs are routinely and continuously adminstered in the feed or drinking water of poultry to control coccidiosis. Extensive use of these drugs has led to the emergence of drug resistant strains and the need for continual development of new, effective drugs. Trigg (1967b) and Norton (1967a; 1967b) have tested the efficacy of several drugs against pheasant coccidia commonly found in Great Britain. However, none of the pheasant coccidial species occuring in the U.S. have been examined for drug sensitivity.

Furazolidone, sulfaquinoxaline and amprolium are widely used drugs introduced in the 1950's and 1960's to control poultry coccidiosis. Because of their extended and wide spread use, drug resistant strains of poultry coccidia are prevalent. As a result, furazolidone and sulfaquinoxaline which are still used as poultry feed additives have had their value limited primarily to anti-bacterial applications. Amprolium, the only drug approved by the Food and Drug Administration as a prophylactic measure against coccidiosis in pheasants, is also becoming obsolete (McDougald, 1982). Since these drugs are routinely added to commercial poultry feeds in the U.S. and these rations are often fed to pheasants, especially among small producers, the knowledge of their efficacy on U.S. strains of pheasant coccidia is important.

This study was designed for two purposes. The first objective was to isolate and identify the coccidial parasites infecting ring-necked pheasants at a state game farm in Illinois. The second objective was to determine the coccidiostatic and coccidiocidal effect of 2-sulfanilamidoquinoxaline (sulfaquinoxaline), 1-(4-amino-2-N-propyl-5-pyrimidinyl methyl)-2-picolinium chloride hydrochloride (amprolium) and 3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone (furazolidone) on the coccidial species found at the game farm.

MATERIALS AND METHODS

Collection and Identification of Parasites

Litter samples were taken from floor pens housing birds 3-4 weeks old at the Glen Palmer State Game Farm at Yorkville, Illinois. The birds were fed non-medicated feed and water ad libitum. The samples were mixed with 2.5% (w/v) aqueous $K_2Cr_2O_7$ and allowed to soak for 0.5h., before being filtered through No. 20, 40, and 60 mesh brass sieves. The solution was placed in a flask and air was pumped through it for seven days.

To determine the concentration of the various species of coccidia in the $K_2Cr_2O_7$ solution, 1 ml of the solution was mixed with 9 ml of saturated sugar solution. The $K_2Cr_2O_7$-sugar solution was mixed for one minute in a vortex mixer and a portion of the solution was used to fill a McMaster's counting chamber. After waiting 15 minutes to allow the oocysts to come to the top of the chamber, the oocysts

were identified and counted with a differential counter. Absolute numbers of the various coccidial species per 1 ml of actual oocyst-$K_2Cr_2O_7$ solution were calculated.

All oocysts measurements were made with a calibrated ocular micrometer on a phase contrast microscope equipped with an achromatic oil immersion objective.

Preparation of Inoculum

The filtered $K_2Cr_2O_7$ solution containing sporulated oocysts from litter samples was centrifuged (450g for 10 min) to remove the $K_2Cr_2O_7$ and diluted with distilled water to obtain 50,000 oocysts/ml. One milliliter was inoculated *per os* into 10 three wk old, parasite-free pheasants to produce a sufficient inoculum for use in drug efficacy studies.

The birds were given non-medicated Illinois pheasant-quail starter ration (Ralston Purina Co., St. Louis, Mo.) and water *ad libitum* and placed in clean, sanitized suspended cages (60 cm × 60 cm × 85 cm). The feces were collected daily in drop pans containing 2.5% (w/v) $K_2Cr_2O_7$ from Day 5 to Day 8 post-inoculation (PI). The solutions were filtered and aerated for seven days at room temperature (22°C).

Differential oocysts counts were determined for each day (Day 5 PI to Day 8 PI) with the use of a McMaster's chamber. The oocyst -$K_2Cr_2O_7$ solutions representing collections from Day 5 PI through Day 8 PI were pooled, centrifuged, the $K_2Cr_2O_7$ removed and distilled water added to obtain a concentration of 50,000 sporulated oocysts of *E. phasiani*, 40,000 oocysts of *E. pacifica*, 140,000 oocysts of *E. duodenalis* and 25,000 oocysts of *E. tetartooimia*. The inoculum was used one wk after preparation.

Experimental Design

Three wk old, female, parasite-free pheasants hatched from an incubator and maintained on nonmedicated Illinois pheasant-quail starter ration were randomly placed in four groups with seven birds per group. Each group had approximately equal total body weights and was housed in suspension cages (60 cm x 60 cm x 85 cm) made of 13mm (0.5 inch) square wire mesh. The cages were kept 30 cm apart in a room continuously lighted and maintained at 32°C. Each group had its own feeder and water fountain. One group was medicated with 0.025% sulfaquinoxaline (Sulfa-Nox®, Ralston-Purina Co., St. Louis, Mo.) in the drinking water, another group received 0.0125% amprolium (Amprol®, Merck & Co., Inc., Rahway, N.J.) in the feed ration and another group received 0.011% furazolidone (NF-180®, Hess & Clark, Inc., Ashland, Ohio) in the feed. All drug concentrations used were manufacturer's suggested or officially approved for poultry. The fourth group served as a control and had no medication in the feed or water throughout the experiment.

Two days after initiation of medication, all the birds were inoculated *per os* with 1 ml of mixed species, sporulated oocyst inoculum.

From Day 5 to Day 15 PI the feces from each group was collected in drop pans containing 2.5% (w/v) $K_2Cr_2O_7$. Oocysts production was determined by homogenizing the sample in a Waring blender for 10 seconds, sieving it and measuring the volume in a graduated cylinder. After a differenital count of oocysts per

ml was determined in a McMaster's chamber, the daily oocyst discharge/bird was calculated. On Day 8 PI all medication was withdrawn and replaced thereafter with non-medicated pheasant-quail ration and tap water *ad libitum*. Mortality was monitored daily and post mortems were conducted to determine if death resulted from coccidial infection. Birds were weighed on Day 15 PI and compared to initial body weights to determine average weight gains. The Student's T-test was used to compare average body weights and weight gains.

RESULTS

Coccidian Species Found

Four coccidian species were identified from the pooled litter samples obtained at the Glen Palmer Game Farm. *Eimeria duodenalis* was the most prevalent, with 57.5% of the oocysts recovered. *Eimeria tetartooimia* represented 24.9% of the coccidial population, while *E. phasiani* and *E. pacifica* had about equal numbers of oocysts totalling 17.6%.

Antibiotic Efficacy vs. Coccidian Species

Eimeria duodenalis and *E. tetartooimia* were the first species to appear in substantial numbers on Day 5 PI in the nonmedicated birds (Fig. 1). By Day 6 PI all four coccidial species were present in the feces at a rate of over 5 million parasites/bird. On day 7 PI, *E. pacifica* peaked with 40.5 million oocysts/bird, *E. phasiani* peaked with 36.8 million oocysts/bird and *E. duodenalis* peaked with 19.4 million oocysts/bird. *E. tetartooimia* remained steady with 6.2 million oocysts/bird. After Day 7 PI all four species showed decreasing numbers of oocysts in the feces. Five of the seven birds died of coccidial infection (71.4%) between Day 5 PI and Day 8 PI.

All four coccidial species appeared in the feces of furazolidone medicated birds on Day 5 PI. *Eimeria tetartooimia* was the most abundant parasite with 4.5 million oocysts/bird (Fig. 2). However, *E. tetartooimia* showed decreasing numbers of oocysts from its peak on Day 5 PI until no oocysts were found after Day 11 PI. In contrast, *E. phasiani*, *E. duodenalis* and *E. pacifica* peaked on Day 7 with *E. pacifica* having the greatest number of oocysts (18 millioin oocysts/bird). *E. phasiani* and *E. duodenalis* peaked with 10.1 and 6.9 million respectively. No significant increase in oocyst discharge was observed with any of the species after furazolidone was withdrawn from the feed on Day 8 PI suggesting that furazolidone had no coccidiostatic action on the parasites. Three birds died of coccidia between Day 5 PI and Day 7 PI.

Amprolium appeared to have a differential effect on the various coccidial species. *Eimeria phasiani* and *E. pacifica* oocyst discharges were much lower than in the furazolidone treated group, but *E. duodenalis* and *E. tetartooimia* were relatively high in comparison (Fig. 3). *E. tetartooimia* had oocyst discharge levels three times higher than the nonmedicated or furazolidone group on Day 5 PI, possibly because amprolium prevented the development of a competitive species in the host. *Eimeria duodenalis* represented the greatest number of oocysts in the fecal samples on Day 7 PI. However, there was no mortality throughout the testing period, suggesting that *E. tetartooimia* and *E. duodenalis* are relatively less pathogenic than

E. phasiani and *E. pacifica*. The secondary peak in oocyst discharge after drug withdrawal on Day 13 PI indicates that amprolium has a partial coccidiostatic effect on *E. pacifica* and *E. phasiani* (Fig. 3).

Sulfaquinoxaline clearly demonstrated a coccidiostatic effect in all the coccidial species infecting the pheasants (Fig. 4). No oocysts of any species were found until Day 12 PI (four days after drug withdrawal). However, the medication also had a predominantly coccidiocidal effect resulting in low oocyst discharge and no mortality. Furthermore, sulfaquinoxaline and amprolium medicated birds had significantly higher body weights and weight gains compared to non-medicated and furazolidone medicated birds (Table 1).

DISCUSSION

Although furazolidone had a coccidiocidal action on the parasite isolates, it did not reduce the coccidia sufficiently to prevent mortality. Furazolidone was the only drug tested which failed to prevent mortality and the average weight gain of furazolidone treated birds was poor compared to amprolium and sulfaquinoxaline medicated birds. Perhaps the extended use of furazolidone in poultry rations and the use of these rations in pheasant production has resulted in furazolidone resistant pheasant coccidia.

Amprolium is officially approved as a feed additive for the prophylactic control of coccidiosis in pheasants at 0.0175% concentration (Anonymous, 1985). This is a slightly greater concentration of amprolium than was tested in this study or that is approved as a feed additive in chicken feed ration (0.0125%). When compared to furazolidone, it appears that amprolium is effective in controlling mortality and maintaining good weight gain in pheasants by effectively reducing the reproductive potential of *E. phasiani*, a known pathogen, and possibly *E. pacifica* (Trigg, 1967a; Trigg, 1967b). The fact that amprolium treated pheasants had no mortality and high weight gains dispite the highest discharge of *E. tetartooimia*, suggests that *E. tetartooimia* is low in pathogenicity. Also, *E. duodenalis* apparently is relatively non pathogenic because it produced relatively high levels of oocysts in amprolium treated birds compared to virtually no oocyst production in sulfaquinoxaline treated birds, yet both groups had comparable weight gains and no mortality (Norton, 1967b). Furthermore, birds medicated with amprolium are also producing a natural immunity to the various coccidial species because each species is allowed to complete its life cycle and produce some oocysts.

The total inhibition of pheasant coccidia by sulfaquinoxaline coupled with its antibacterial action and water solubility are attractive characteristics for its use in pheasant operations. However, drug toxicity in poultry from prolonged use or administration of high dosages (Cuckler and Ott, 1947; Joyner et al, 1963) requires the prudent use of sulfaquinoxaline.

ACKNOWLEDGMENTS

The authors thank Mr. Robert Bauer, Program Manager of Game Propagation of the Illlinois Department of Conservation for supplying the pheasants and feed for the study and Mr. Michael Brady, Manager of the Glen Palmer State Game Farm, Yorkville, Illinois for his technical assistance.

LITERATURE CITED

Anonymous. May 1, 1985. Department of Health and Human Services, Food and Drug Administration. Tolerances for Residues of New Animal Drugs in Food; New Animal Drugs for use in Animal Feeds; Amprolium for Pheasants. *Federal Register*. 50(84):18471-18472.

Cuckler, A.C. and W.H. Ott. 1947. The effect of sulfaquinoxaline on the developmental stages of *Eimeria tenella*. J. Parasitol. 33 (suppl.):10.

Fisher, J.W. and R.S. Wacha. 1976. Coccidian parasites from game-farm reared pheasants, *Phasianus colchicus*, in Iowa. Proc. Helminthol. Soc. Wash. 43(2):226-227.

Haase, A. 1939. Untersuchangen uber die kie deutscher Wildhuhnern vorkommenden *Eimeria*-Arten. Arch. Protistenk. 92:329-383.

Joyner, L.P., S.E.M. Davies, and S.B. Kendall. 1963. Chemotherapy of Coccidiosis. *In* R.J. Schnitzer and F. Hawking (eds.), *Experimental Chemotherapy*. Vol. 1, pp. 445-486. Academic Press, New York.

McDougald, L.R. 1982. Chemotherapy of Coccidiosis *In* P.L. Long, (ed.). *The Biology of The Coccidia*. pp. 373-427. University Park Press, Baltimore.

Norton, C.C. 1967a. *Eimeria colchici* sp. nov. (Protozoa: Eimeriidae), the cause of cecal coccidiosis in English covert pheasants. J. Protozool. 14:772-781.

Norton, CC. 1967b. Eimeria duodenalis sp. nov. from English covert pheasants *(Phasianus* sp.). Parasitology. 57:31-46.

Ormsbee, R.A. 1939. Field studies in coccidiosis in the ring-necked pheasants of Eastern Washington. Parasitology. 31:389-399.

Todd, K.S. Jr. and D.M. Hammond. 1971. Coccidia of Anseriformes, Galliformes and Passeriformes *In* J.W. Davis, R.C. Anderson, L. Karstad and D.O. Trainer, eds., *Infectious and Parasitic Diseases of Wild Birds*. The Iowa State University Press, Ames, pp. 234-280.

Trigg, P.I. 1967a. *Eimeria phasiani* Tyzzer, 1929 - a coccidium from the pheasant *(Phasianus colchicus)*. The life cycle. Parasitology. 57:135-145.

Trigg, P.I. 1967b. Eimeria phasiani Tyzzer, 1929 - a coccidium from the pheasant *(Phasianus colchicus)*. II. Pathogenicity and drug action. Parasitology. 57:147-155.

Tyzzer, E.E. 1929. Coccidiosis in gallinaceous birds. Am. J. Hyg. 10:269-383.

Wacha, R.S. 1973. The sporulated oocysts of *Eimeria tetartooimia* sp. n., and *E. duodenalis* and *E. pacifica* (Protozoa:Eimeriidae) redescribed, from the ring-necked pheasant, *Phasianus colchicus*. Proc. Helminthol. Soc. Wash. 40:56-58.

Yakimoff, W.L. and S.N Matschoulsky. 1937. Nouvelle coccidie due faisen. Ann. Parasitol. 15:162-163.

Table 1. Average Body Weights and Weight Gains of Three Week-Old Pheasants Medicated With Antibiotics and Infected With Coccidia.

Average Body Weights (grams)	Treatment (7 birds/group)[A]			
	Non-Medicated	Furazolidone	Amprolium	Sulfa quinoxaline
Initial Weight	96.69	95.59	96.16	94.85
Final Weight (Including Dead Birds)	133.33a	176.33a	247.54b	252.93b
Weight Gain	36.64a	80.74a	151.38b	158.08b

A. Averages within a row not followed by the same letter are significantly different ($P<0.05$).

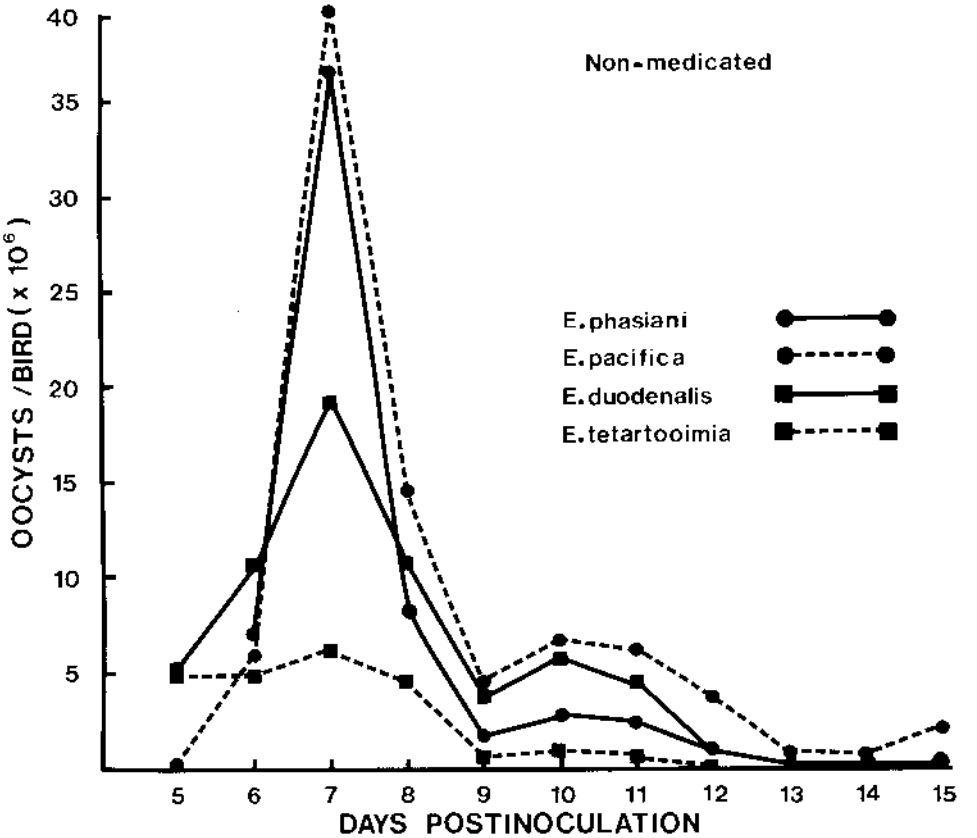

Fig. 1. Average daily oocyst discharge of three week-old ring-necked pheasants inoculated with a mixed culture of coccidian parasites previously isolated from a game farm.

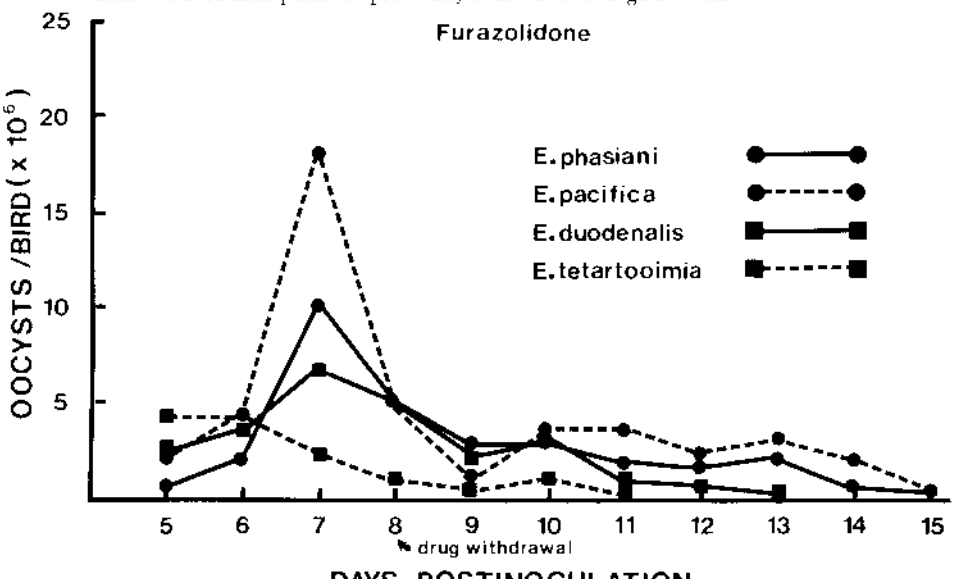

Fig. 2. Average daily oocyst discharge of furazolidone medicated, three week-old ring-necked pheasants inoculated with a mixed culture of coccidian parasites previously isolated from a game farm.

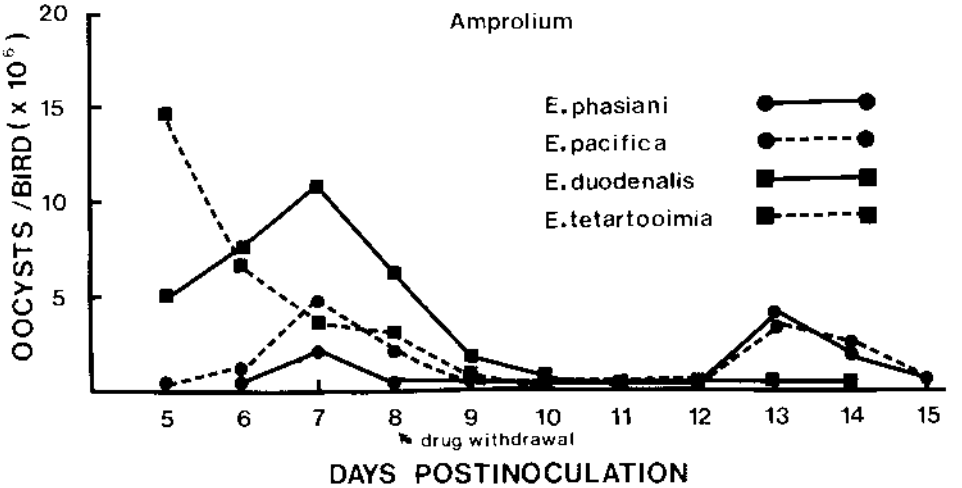

Fig. 3. Average daily oocyst discharge of amprolium medicated, three week-old ring-necked pheasants inoculated with a mixed culture of coccidian parasites previously from a game farm.

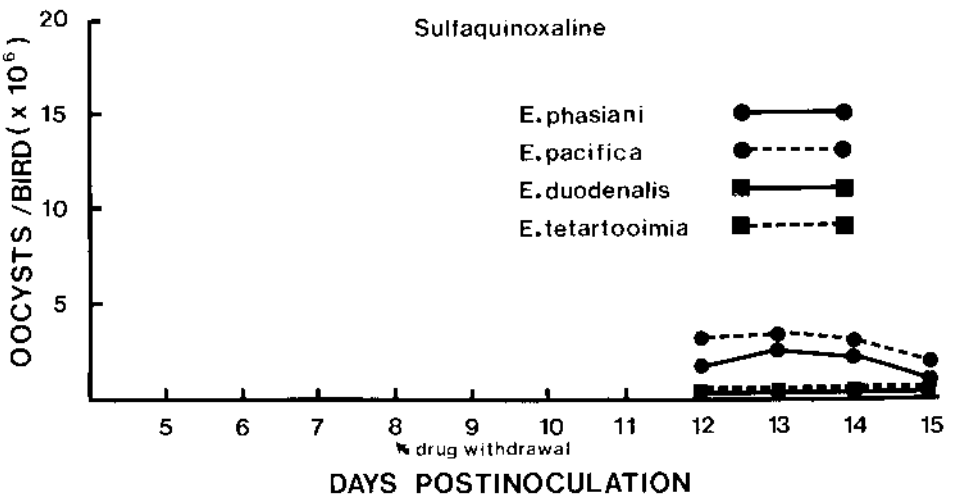

Fig. 4. Average daily oocyst discharge of sulfaquinoxaline medicated, three-week old ring-necked pheasants inoculated with a mixed culture of coccidian parasites previously isolated from a game farm.