

The Anticoccidial Effects of Amprolium, Monensin and Sodium Sulfamethazine in Farm-Reared Chukar Partridges (*Alectoris graeca*) in Illinois

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ABSTRACT

Coccidian parasites were isolated from litter samples and intestinal contents of chukar partridges from a game farm and inoculated into parasite free three week old partridges medicated with amprolium, monensin and sodium sulfamethazine to determine efficacy against the parasite isolates. Amprolium exhibited no coccidiostatic activity and moderate reduction of oocyst discharge but did not prevent mortality. Monensin showed little coccidiocidal and no coccidiostatic effect against the parasites, but it did prevent mortality and morbidity as indicated by comparable body weight gains with non-infected controls. Sulfamethazine showed complete coccidiostatic activity and was moderately effective in reducing oocyst discharge while preventing mortality and minimizing morbidity.

INTRODUCTION

Chukar partridges are popular game birds and hundreds of them are raised on game preserves throughout the United States for hunting and food. The methods employed in raising partridges are very similar to those used in the poultry industry. Coccidiosis and other parasitic diseases familiar to the poultry farmer are prevalent on game farms (McQuiston and Dingman, 1986; McQuiston, 1987) causing weight loss, poor feed conversion and mortality in game birds like chukar partridges.

Six coccidial species have been described from the chukar genus *Alectoris*, however, only three species have been confirmed. The other three species are considered synonyms (Levine, 1988). *Eimeria alectoreae* (Ray and Hiregauder, 1959) was reported from India. *Eimeria kofoidi* (Yakimoff and Matikaschwili, 1936), and *E. caucasica* (Yakimoff and Buewitsch, 1932) were reported in Russia. It is not surprising that all of the coccidian species were discovered in Central Asia, the native habitat of the chukar partridge.

Anticoccidial drugs are routinely and continuously administered in the feed or drinking water of birds raised in the poultry industry to control coccidiosis; however, medication to prevent or control coccidiosis in chukars has not been documented. Three anticoccidial drugs that are readily available and used in poultry are amprolium, monensin and sodium sulfamethazine. Since these drugs are routinely added to commercial poultry feeds in the

U. S. and these poultry rations are often fed to chukars, especially among small producers, the knowledge of their efficacy on U. S. strains of chukar coccidia is important.

Amprolium was introduced in 1960 and is one of the safest anticoccidial drugs to be used extensively in the poultry industry, because it can be fed at several times its recommended dose with no ill effects (McDougald, 1982). By 1966, drug resistant strains of coccidia became a problem, so amprolium was combined with other drugs to extend and strengthen its spectrum of activity. Amprolium is still routinely added to poultry feed today.

Monensin was the first polyether ionophorous antibiotic used in commercial poultry feed, and it became a successful antibiotic in controlling morbidity and mortality from coccidiosis in poultry. Although some coccidian resistance to monensin has been reported since 1985 (Ruff et al., 1985; McDougald et al., 1986), monensin continues to be used in poultry rations.

Sulfamethazine, a sulfonamide, has a broad spectrum of activity against *Eimeria* species, despite reports of sulfonamide resistant strains of coccidia as early as 1954 (Waletzky et al., 1954). Their broad action on most endogenous stages of the avian coccidian life cycle and their water solubility have made sulfonamides an important treatment of established coccidial infections (Joyner et al., 1963).

This study was designed for two purposes. The first objective was to isolate and identify the coccidial parasites infecting chukar partridges at a private game farm in southern Illinois. The second objective was to determine the coccidiostatic and coccidiocidal effect of amprolium, monensin and sulfamethazine on the coccidian parasites isolated at the game farm.

MATERIALS AND METHODS

Collection and Identification of Parasites

Litter samples were collected from floor pens housing 3-4 week old birds from a privately owned game farm at Neoga, Illinois. 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 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 1ml of actual oocyst- $K_2Cr_2O_7$ solution were calculated.

All oocyst 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 15,000 oocysts/ml. One milliliter was inoculated *per os* into 20 three week old, parasite-free chukar partridges to produce a sufficient inoculum for use in drug efficacy studies.

The birds were given non-medicated Purina® game bird ration and water *ad libitum* and placed in clean, sanitized suspended wire mesh cages. The feces were collected daily in drop pans containing 2.5% $K_2Cr_2O_7$ from Day 5 to Day 10 post-inoculation (PI). The solutions were blended, filtered and aerated for 7 days at room temperature.

The pooled $K_2Cr_2O_7$ solution representing the oocyst collection from Day 5-10 PI was centrifuged, the $K_2Cr_2O_7$ removed and distilled water added to obtain a concentration of 15,000 oocysts/ml. The inoculum was used immediately after preparation.

Experimental Design

Four week old, parasite-free chukar partridges hatched from an incubator and maintained on non-medicated Purina® game bird ration were randomly placed in five groups with five birds per group. Each group had approximately equal body weights and was housed in suspension cages 60 cm x 60 cm x 30 cm) made of 13mm (0.5 inch) square wire mesh. The cages were kept in a continuously lighted room and maintained at 25° C. Each group had its own feeder and water fountain. One group was medicated with 0.0125% amprolium (Amprol®, Merck & Co., Inc., Rahway, N.J.) in the feed ration. Another group received medicated feed with 0.0120% monensin (Coban-45®, Elanco Products Co., Indianapolis, Indiana) and a third group received .0970% sodium sulfamthazine (Sulmet®, American Cyanamid Co., Wayne, N.J.) in the drinking water. 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. The fifth group served as a non-infected control and was neither infected nor received medication in the feed or water throughout the experiment.

Two days after initiation of medication, all birds, with the exception of the non-infected control group, were inoculated *per os* with 1 ml of sporulated oocyst inoculum containing an estimated 15,000 oocysts.

From Day 5 to Day 15 post inoculation (PI) the feces from each infected group was collected in drop pans. Oocyst production was determined by mixing the feces with 2.5% $K_2Cr_2O_7$, homogenizing the sample in a blender for 10 seconds, and measuring the volume in a graduated cylinder. Volumes were recorded and two samples were placed in labelled vials and stored in the refrigerator for determining parasite concentration later. The remaining fecal/ $K_2Cr_2O_7$ mixture was discarded. After a 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 game bird ration and tap water *ad libitum*. Mortality was monitored daily and *post mortems* were conducted to determine if death resulted from coccidial infection. Fecal samples from the nonmedicated noninfected control group were examined periodically for oocysts

during the experiment. Birds were weighed on Day 16 PI and compared to initial body weights to determine the average weight gains.

RESULTS

Coccidian Species Found

Two coccidian species were identified from the pooled litter samples obtained from the game farm. *Eimeria kofoidi* was the most prevalent, with 59% of the oocysts recovered and *Eimeria alectorae* represented 29.5% of the coccidian population. A third unidentified population of oocysts, believed to be a new species, totalled 11.5%.

Antibiotic Efficacy

Chukar partridges in the non-medicated infected group began passing oocysts on Day 5 PI (Fig. 1). By Day 6 PI, oocysts were present in the feces at a rate of over 12 million oocyst/bird. On day 7 PI, oocyst discharge peaked with 14 million oocyst/bird and then began to decline thereafter. Two of the five birds died of coccidial infection between Day 8 PI and Day 10 PI.

The amprolium medicated birds began passing oocysts on Day 5 PI and the oocyst output steadily increased until it peaked on Day 10 PI with 5 million oocysts/bird and then gradually decreased (Fig. 2). One bird died from coccidial infection on Day 11 PI. Although amprolium reduced the overall oocyst discharge compared to the nonmedicated infected group, it did not prevent mortality or morbidity (Table 1). Amprolium had no coccidiostatic effect on the parasites as indicated by a lack of a secondary peak in oocyst output after Day 8 PI when the medication was withdrawn from the feed ration.

In the monensin medicated group, oocysts were observed on Day 5 PI in relatively small numbers, but increased significantly on Day 6 PI and continued to be observed in the feces until the end of the collection period (Fig. 3). Like amprolium, monensin had no coccidiostatic effect on the parasite isolates and appeared to be less effective in reducing oocyst discharge compared to amprolium. There was no mortality in the monensin medicated group and there was no difference in weight gain compared to the nonmedicated noninfected control group.

Sulfamethazine medicated birds exhibited the most significant suppression of oocyst discharge. Yet, after the initial presence of oocyst in the feces on Day 10 PI, a significant number were observed on Days 13 and 14 PI (Fig. 4). Oocyst presence in the feces on Day 10 PI in high numbers suggests that sulfamethazine had primarily a coccidiostatic action on the parasites because once the medication was withdrawn on Day 8 PI, the infection was allowed to run its course in the host.

The nonmedicated noninfected control group did not pass any oocysts during the entire experiment. The monensin and sulfamethazine medicated groups had comparable weight gains to the nonmedicated noninfected control group (Table 1).

DISCUSSION

Although amprolium had some coccidiocidal action, by reducing the number of oocysts released, it did not reduce the coccidia sufficiently to prevent mortality. Amprolium was the only drug tested which failed to prevent mortality and the average weight gain of amprolium treated birds was poor compared to monensin and sulfamethazine medicated birds. Because of its extended and wide spread use, amprolium resistant strains of poultry coccidia are prevalent, and as a result, the drug is becoming obsolete (McDougald, 1982). It is apparent from this study that amprolium resistant chukar coccidia has developed as well. In fact, the game farm has routinely used amprolium medicated feed for their chukar flocks in the past.

Monensin is documented to be one of the most successful antibiotics in controlling morbidity and mortality in domestic poultry. Research results suggest that monensin does not effect the sporulation of the oocysts passed by the birds infected with eimerian parasites, but does reduce the pathogenic effects caused by oocyst production. (Joyner and Norton, 1977). When compared to amprolium and the nonmedicated infected group, it appears that monensin is effective in controlling mortality and maintaining good weight gain in chukars by effectively reducing the pathogenicity of the coccidia.

The significant suppression of chukar coccidia by sulfamethazine (Sulmet®) coupled with its water solubility are attractive characteristics for its use in chukar operations. Judging from the coccidiostatic activity of sulfamethazine after the drug was withdrawn from the drinking water, it appears that sulfamethazine interferes with the life cycle of *Eimeria* as early as the first schizontic generation, no later than the second, and suppressed the ultimate formation and discharge of oocysts. However, once medication is removed from the water supply the life cycle resumes at the point of suppression. While sulfamethazine did appear to have some depression of weight gains, perhaps due to drug toxicity, it was not an appreciable amount when compared to the nonmedicated infected and amprolium medicated birds. However, drug toxicity in poultry from prolonged use or administration of high dosages (Joyner et al, 1963) requires the prudent use of sulfamethazine.

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Figure 1. Average daily oocyst discharge of three week old chukar partridges inoculated with a culture of *Eimeria* parasites previously isolated from a game farm.

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Figure 2. Average daily oocyst discharge of amprolium medicated, three week old chukar partridges inoculated with a culture of *Eimeria* parasites previously isolated from a game farm.

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Figure 3. Average daily oocyst discharge of monensin medicated, three week old chukar partridges inoculated with a culture of *Eimeria* parasites previously isolated from a game farm.

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Figure 4. Average daily oocyst discharge of sulfamethazine medicated, three week old chukar partridges inoculated with a culture of *Eimeria* parasites previously isolated from a game farm.

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Table 1. Average body weights and weight gains of chukar partridges medicated at 3 weeks of age and infected with coccidia.

Treatment ^A	Average body wt. (gms.)		Weight gain (gms.)
	Initial	Final	
Nonmedicated, uninfected	128.8	241.2	112.4
Nonmedicated, infected ^B	134.4	206.0	71.6
Amprolium ^B	141.2	219.0	77.8
Monensin	137.6	241.8	104.2
Sulfamethazine	134.2	226.6	92.4

^A Five birds in each group.

^B Averages do not include dead birds.