Restoration of sensitivity to salinomycin in *Eimeria* following 5 flocks of broiler chickens reared in floor-pens using drug programs and vaccination to control coccidiosis

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**ABSTRACT** Five successive flocks of broilers were reared in floor-pens and given different drug programs or were vaccinated against coccidiosis. Oocysts of *Eimeria* were isolated from the litter of pens during the fifth flock and their sensitivity to salinomycin (Sal) investigated by measuring new oocyst production following infection of medicated and unmedicated birds. Parasites obtained following 5 flocks given Sal were not well-controlled and it was concluded that they were partially resistant to the drug. Parasites obtained following 4 unmedicated flocks and one medicated flock were better controlled by Sal and it was concluded that in the absence of continuous medication there had been an improvement in drug efficacy. Sal almost completely suppressed oocyst production of isolates from treatments in which medication was followed by vaccination, indicating that when a drug program is followed by vaccination, restoration of sensitivity to Sal had occurred.

**Key words:** *Eimeria*, broiler, anticoccidial drug, vaccine, coccidiosis

**INTRODUCTION**

Coccidiosis is a disease of livestock caused by protozoan parasites of the genus *Eimeria*. In poultry, outbreaks of coccidiosis characterized by high mortality are uncommon but poor performance, manifest by reduced feed intake, poor weight gain, and impaired feed conversion results in losses to the poultry industry of many millions of dollars per annum (Williams, 1999). In broiler chickens, the disease is principally controlled by the inclusion of anticoccidial drugs in the feed. These can be broadly divided into two categories, synthetic agents produced by chemical synthesis that include compounds such as diclazuril (*Dic*, Clinacox Huvepharma) and the ionophorous antibiotics that are produced by fermentation (ionophores) such as salinomycin (*Sal*: Sacox Huvepharma). The extensive prophylactic use of drugs has resulted in a well-documented loss of parasite sensitivity (drug-resistance) resulting in a decline in efficacy (Chapman, 1997). Various procedures have been adopted to ameliorate resistance such as to alternate the use of compounds with different modes of action. This can take several forms such as the so-called “shuttle” program in which different compounds are incorporated in different feeds provided to birds. Often a synthetic agent such as *Dic* may be incorporated in the first (starter) feed followed by an ionophore such as *Sal* in the second (grower) feed. Another method is the so called “rotation” program in which compounds with different modes of action are employed in successive flocks (McDougald, 1982). An underlying philosophy behind these programs is that if resistant parasites are selected then they will be controlled if a compound with a different mode of action is used. However, evidence to support this contention is anecdotal.

Another approach to the problem of resistance involves the alternation of medication with the use of live vaccines that comprise oocysts of sensitive species of *Eimeria* (Chapman et al., 2002). Coccivac-B (Merck Animal Health) is suitable for this purpose because the component strains were isolated in the 1950s, before the introduction of modern anticoccidial drugs (Chapman, 2000). It is believed that such vaccines may repopulate poultry houses with sensitive strains and that this will result in a restoration of drug efficacy (Chapman and Jeffers, 2014). Although restoration of drug efficacy has been demonstrated in the field, both in chickens and turkeys (Chapman, 1994; Mathis and Broussard, 2006; Mathis and McDougald, 1989; Peek and Landman, 2003), experimental evidence in which birds are raised under simulated field conditions using practical coccidiosis control programs is lacking. In this study 5 successive flocks of broilers were raised in floor-pens in which different control programs were employed. The objective was to determine whether a change in
the sensitivity of the resident coccidia to drugs had occurred.

MATERIALS AND METHODS

Floor-Pen Experiment

Prior to commencement of the floor-pen experiment, “seeder birds” were introduced into the pens in order to provide a uniform background infection. Unvaccinated male broiler chicks (Cobb-Vantress Inc., Siloam Springs, AR) were obtained from a local hatchery and brooded at a stocking density of 257 cm²/poult in a 6-tiered battery unit. Batteries had been thoroughly cleaned and fumigated with concentrated ammonia solution prior to use. At 9 d age they were orally inoculated with 200 oocysts of E. acervulina, 200 oocysts of E. maxima, and 200 oocysts of E. tenella that had been isolated from broiler farms in Arkansas. Methods for their purification and propagation have been described by Shirley (1995). A drug sensitivity test was previously performed which indicated that they were partially resistant to Sal and Dic (Chapman, unpublished observations). The birds were then transferred to 32 floor-pens (each 5.2 m²) that contained new wood shavings to provide about 10 cm absorbent dry litter, 2 tubular hanging feeders, and an automatic bell-type water font. Five seeder birds were allocated to each pen and given an unmedicated starter feed. Eight d later they were removed by which time they had shed fresh oocysts onto the litter.

Two d after removal of the seeder birds, 1,216 newly hatched chicks were obtained from the same hatchery and randomly allocated to the pens (38 chicks/pen) at a stocking density of 0.14 m²/chick. They were given 3 feeds, a starter (S) from d 0 to 21, a grower (G) from d 21 to 42, and an unmedicated finisher from d 42 to 49. The basal diet was a corn/soybean meal formulation that met the nutritional requirements of the broiler chicken (National Research Council, 1994). Medicated treatments were given Sal or Dic incorporated in the feed at concentrations of 66 and 1 ppm, respectively. Birds in vaccinated treatments were infected by spraying Coccivac-B at the dose recommended by the manufacturer on a portion of feed placed on a feed tray soon after chicks were placed on the litter. Feeders were raised 2 h before vaccination to ensure feed containing the vaccinal oocysts was consumed. The interval between successive flocks (down-time) was 7 d and during this period any cake beneath water-fonts was removed and a top dressing of new litter applied.

Study Design

The study design is shown in Table 1. There were 4 treatments, each comprising 8 pens of birds. A randomized block design was used for the first flock and in subsequent flocks treatments were assigned to the same pens. Treatments were as follows: 1) 5 flocks given Sal in S and G feeds, 2) 4 unmedicated flocks plus one flock given Sal, 3) 2 flocks given Sal in S and G feeds followed by 2 vaccinated flocks and one Sal flock, and 4) 2 flocks given Dic in the S and Sal in the G feed followed by 2 vaccinated flocks and one Sal flock.

Sensitivity Test

When birds in the fifth flock were four weeks old, a sample of surface litter (approximately 100 g) was taken from each pen. Samples from each treatment (8 pens) were combined and then processed in the laboratory to isolate any oocysts of Eimeria present. Few oocysts could be recovered from the litter of Treatments 3 and 4. Oocysts were sporulated as described by Shirley (1995) and then used in a sensitivity test to determine their response to 66 ppm Sal. Insufficient oocysts could be recovered to determine the species of Eimeria present or their sensitivity to Dic.

One hundred and sixty chicks were raised in a brooder until they were 9 d age and then randomly allocated to 32 cages in a battery unit (5 birds/cage). The design of the study is given in Table 2. There were 8 treatments each comprising 4 replicate cages. Treatments A1, B1, C1, and D1 were given Sal in the feed and Treatments A2, B2, C2, and D2 were unmedicated. Two d after they were assigned to cages, Treatments A1 and A2, B1 and B2, C1 and C2, and D1 and D2 were infected with 500 oocysts obtained from Treatments 1 to 4 of the floor pen study, respectively. Feces were collected from the trays in each cage from d 5 to 8 following

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The table below provides the experimental design for the floor-pen study.

**Table 1. Experimental design, floor-pen study.**

<table>
<thead>
<tr>
<th>Flock</th>
<th>Treatment</th>
<th>Medication</th>
<th>Infection source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sal</td>
<td>Sal</td>
<td>Trt 1</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>None</td>
<td>Trt 1</td>
</tr>
<tr>
<td>3</td>
<td>Sal</td>
<td>None</td>
<td>Trt 3</td>
</tr>
<tr>
<td>4</td>
<td>Dic</td>
<td>None</td>
<td>Trt 3</td>
</tr>
<tr>
<td>5</td>
<td>Sal</td>
<td>Sal</td>
<td>Trt 4</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>None</td>
<td>Trt 4</td>
</tr>
<tr>
<td>7</td>
<td>Sal</td>
<td>Sal</td>
<td>Trt 4</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>None</td>
<td>Trt 4</td>
</tr>
</tbody>
</table>

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The table below provides the experimental design for the sensitivity study.

**Table 2. Experimental design, sensitivity study.**

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Infection source</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Sal</td>
<td>Trt 1</td>
</tr>
<tr>
<td>A2</td>
<td>None</td>
<td>Trt 1</td>
</tr>
<tr>
<td>B1</td>
<td>Sal</td>
<td>Trt 2</td>
</tr>
<tr>
<td>B2</td>
<td>None</td>
<td>Trt 2</td>
</tr>
<tr>
<td>C1</td>
<td>Sal</td>
<td>Trt 3</td>
</tr>
<tr>
<td>C2</td>
<td>None</td>
<td>Trt 3</td>
</tr>
<tr>
<td>D1</td>
<td>Sal</td>
<td>Trt 4</td>
</tr>
<tr>
<td>D2</td>
<td>None</td>
<td>Trt 4</td>
</tr>
</tbody>
</table>

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1For each treatment (trt), 4 replicate cages, each containing 5 birds, were infected with isolates of Eimeria obtained from Trt 1–4 of the floor-pen study. Sal = Salinomycin.
The objective of this study was to investigate whether sensitivity to anticoccidial drugs could be restored following 5 successive flocks in floor-pens in which birds were given different drug programs and vaccinated against coccidiosis. Prior to commencement, a uniform background infection in the pens was achieved by introducing oocysts of Eimeria into the litter using seeder birds, as described by Callender and Tonkinson (1971). Seeder birds were infected with a mixture of E. acervulina, E. maxima, and E. tenella, species that are widespread in broiler flocks (Jenkins et al., 2010). The isolates used were partially resistant to Sal and Dic (Chapman, unpublished observations). Resistance to these drugs is fairly typical of isolates obtained from commercial broiler flocks in the United States (e.g. Chapman and Hacker, 1994; Chapman and Shirley, 1989; Mathis, 1999; Mathis and Broussard, 2006; Mathis et al., 1984).

The sensitivity of isolates of Eimeria was determined in a battery experiment by obtaining oocysts from the litter of pens when birds of the fifth flock were 4 wk age. Very few oocysts could be recovered from the litter of birds from Treatments 3 and 4 and insufficient numbers were available to investigate whether sensitivity had been restored to Dic. Drug resistance was measured by counting oocyst numbers in the feces following infection with a low dose (500) of oocysts. Oocyst counts provide a measure of the ability of Eimeria species to multiply in chickens receiving medication if small doses (<1,000 oocysts/bird) are administered, and provide one criterion for evaluating drug sensitivity (Chapman, 1998). Such counts are particularly useful where only a few parasites are available, as was the case in this study. It would be possible to obtain larger numbers by propagating recovered oocysts in unmedicated birds but it was considered important to evaluate drug sensitivity of parasites obtained directly from the pens of medicated groups. Low numbers of oocysts likely reflect the magnitude of challenge that can occur in the field rather than the large doses often administered in drug sensitivity tests (Chapman, 1999). Indeed, some workers have questioned the validity of sensitivity studies where large doses of oocysts are administered (Watkins, 1997).

The results indicate that parasites from the treatment in which birds were given Sal for 5 flocks (Treatment 1) were not well-controlled by this drug. It is concluded that continuous medication with an ionophore in a single drug program is likely to perpetuate resistance to this class of drug. Fewer oocysts were produced by medicated birds given oocysts from Treatment 2 suggesting that in the absence of medication there had been a partial restoration of drug sensitivity. Experimental studies have indicated that resistance to ionophores is stable following propagation of parasites in unmedicated chickens although there are contradictory observations (Chapman, 1984, 1986a,b; Jeffers, 1989). However, loss of resistance may occur if resistant parasites are diluted by the introduction of drug-sensitive strains (Jeffers, 1976; Long et al., 1985). One possibility is that drug-sensitive vaccine strains had unintentionally been transferred to the unvaccinated pens of Treatment 2 resulting in a partial restoration of drug sensitivity.

Very few oocysts were produced by medicated birds given isolates obtained from Treatments 3 and 4. It is concluded that these isolates were sensitive to Sal. A probable explanation is that following vaccination the floor-pens had been repopulated with vaccinal strains.
that were inherently drug-sensitive. Loss of resistance following admixture of sensitive and resistant strains has previously been reported (Ball, 1966; McLoughlin and Chute, 1979; Long et al., 1985). Jeffers (1976) proposed that the pattern of drug sensitivity in a population of coccidia can be greatly altered by the introduction of drug-sensitive coccidia and that this can be accomplished through the use of some coccidiosis vaccines. The present results support this proposal. Restoration of sensitivity to ionophores and other drugs has been described from the field (e.g., Chapman, 1994; Jenkins et al., 2010) but has not previously been demonstrated under experimental conditions in floorpens. The results indicate that sensitivity to Sal was restored whether vaccination followed a shuttle program involving a synthetic drug (Dic) and an ionophore (Sal) or a single ionophore program (Sal). Insufficient oocysts were recovered to determine whether sensitivity to Dic was also restored in this study. Restoration of sensitivity to this drug in the field as a result of vaccination has been described by Mathis and Broussard (2006). The present results support the contention that when drug programs are followed by vaccination restoration of drug sensitivity may occur. This may extend the useful life of anticoccidial drugs and provide a sustainable means to control coccidiosis.

REFERENCES


