

Coccidiosis in Game Birds: Part III

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Introduction

Coccidiosis is a major limitation to the production and marketing of game species of birds in America, principally Bobwhite Quail, Ringneck Pheasants, and the Chukar Partridge. Virtually no research has been conducted on the disease agents, other than to describe some of the species, and to conduct tests on the potential for control by existing anticoccidial drugs. Clearly, an organized effort is needed by the industry to generate the technical capability of control of coccidiosis, and to produce a vaccine for more reliable prevention. A research program consisting of 3 parts have been proposed.

First, we identified the responsible disease agents in the three avian species of interest. The coccidia of avian species are host-specific. Each bird has its own species of coccidia. Even closely related species of birds may have separate species of coccidia. Thus, identification of those coccidia species responsible for disease in Bobwhite Quail, Ringneck Pheasants, and the Chukar Partridge is of utmost importance. Secondly, several laboratory tests of existing anticoccidial products were conducted with each of the target bird species against infections with the species of coccidia identified in part I. Additional testing is needed to determine whether the anticoccidials are toxic when used in these particular avian species. And thirdly, the development of vaccines by attenuation of 1 or 2 important species of coccidia in each type of bird might be effectively employed for a more reliable control method. Although this objective is unlikely to be completely accomplished within the budget and time span of this project, the findings from this study will give preliminary data so that vaccination in game birds can be further explored.

Materials and Methods

I. Identification of the responsible disease agents: Coccidia can be generally identified by size and other biological characteristics, so there is no need to develop 'high tech' tests for diagnosis. These coccidia can only be propagated by infection of young birds, so it is necessary to keep groups of birds in laboratory cages for this purpose. When positive isolation is made from the birds, an assessment of the pathogenicity and other characteristics can be accessed. Samples were collected from many different farms and tested to develop a good preliminary basis of which coccidia species are the disease-causing organisms. The cultures obtained in this stage of testing were used in other parts of the project.

II. Testing of anticoccidial drugs: In the short term, several laboratory tests of existing anticoccidial products were conducted with each of the target bird species, against infections with the species of coccidia identified in part I. Additional testing is needed to

determine whether the anticoccidials are toxic when used in these birds. This type of testing is common in chicken or turkey coccidiosis. A typical test includes uninfected controls, infected controls, and infected birds given 3 levels of the test product. The treatments were replicated in 3 or more cages with about 10 birds each, so that there would be sufficient statistical power for the test. The results of the tests were evaluated on the basis of 1) weight gain during the infection period, 2) mortality from coccidiosis, and 3) intestinal lesions of birds sacrificed for necropsy. In some cases, it was an advantage to count the infective organisms in the droppings as an estimation of the extent of disease as an alternative to lesion score. These methods have proved reliable in previous tests with chickens, turkeys, and game birds such as pheasants and chukars (McDougald 2003).

III. Immunization as a Possible Control Program for Gamebirds: Following the developments in the poultry industry, we conducted studies to establish the scientific reliability of protection against coccidiosis in gamebirds by vaccination with a live product. In chickens and turkeys, it is common to vaccinate using only a few oocysts from attenuated or non-attenuated strains of coccidia. Each of the bird species was tested separately. Vaccinations were given by individual oral inoculations when birds were about one week of age. After the birds had time to build an immune response to the vaccination dosage, they were 'challenged' with a dosage of coccidia sufficient to produce severe clinical signs. Results were based on a reduction of clinical signs and improvement of growth within a week after challenge, in comparison with birds that were not vaccinated.

Vaccination of Pheasants: Pheasants were vaccinated with 100, 200 or 1000 coccidia (*E. phasianii*), then challenged with 125,000 or 250,000 coccidia.

Vaccination of Chukars:

1. Chukars were given 100 or 1000 oocysts of two different species of coccidia, the challenged several weeks later.

2. We tested an experimental vaccine (100 oocysts of *E. koffoidi* and 100 of *E. legionensis*), in comparison with a commercial vaccine (Coccivac-T, 1x or 10x) containing *E. dispersa*.

3. After completion of the 2 year study, a pen trial with chukars was conducted to evaluate a vaccination program under simulated practical conditions. Birds were reared in large cages with pine shavings on the floor. Vaccination was given during the first week with Coccivac T or with an experimental vaccine as above. Several weeks after vaccination, birds were challenged with virulent coccidia.

Vaccination of Bobwhite Quail:

1. Quail were given 100 or 1000 oocysts (probably *E. lettyae*) and challenged 4 weeks later.

Results

I. Samples were submitted from outbreaks in 22 states, representing all 3 species of birds. These were propagated and readied for further work in the laboratory. In some instances, isolated coccidia were used in drug tests, or for work with PCR test development. The lesions caused by *Eimeria* in these birds were often severe, but were not distinctive in differentiation of species. Typically, severe infections of coccidiosis caused mucus and fluid in the gut, often from the duodenum to the ceca, sometimes with white cecal cores, regardless of apparent species. This emphasizes the need for further work on PCR to confirm suspected diagnosis of species. The isolates received and processed are listed in Table 1.

Table 1. Coccidia species found in field isolates during the study.

Host bird	Predominant coccidia
Ringneck pheasant	<i>Eimeria colchici</i> *, <i>E. phasiani</i> *, <i>E. duodenalis</i> , (and others, including <i>E. tetartoomia</i> , and <i>E. pacifica</i>)
Chukar Partridge	<i>E. kofoidi</i> *, <i>E. legionensis</i> *
Bobwhite Quail	<i>E. lettyae</i> *, <i>E. dispersa</i> , <i>E. colini</i>

* Judged highly pathogenic

II. Tests of anticoccidial drugs: The parameters used in this series of tests were similar to those used for this type of work in chickens. Anticoccidials are evaluated on the basis of protection against lesions and diarrhea (lesion scores and fecal scores), and improvement in weight gain relative to the unmedicated, infected controls (NMI). However, it should be cautioned that lesion scores are far more subjective in these tests, and probably less reliable, because a satisfactory system of grading lesion scores has not been established for game birds. The lesions seen in gamebirds infected with *Eimeria* lack the specificity we expect in chickens, and are not as easily graded. Thus, our system was based on a 3 point scale, where 1=mild lesions, 2=moderate lesions, or 3=severe lesions. Lesions of all species looked fairly similar and were characterized by the presence of mucus and fluids. Following the second trial, we added another observation, fecal score, which is simply a subjective observation of the severity of diarrhea. Trials were performed with averages of 4 replicate cages of 5 birds each. Birds were given medicated feed for 2 days before inoculation with measured dose of coccidia. Test was terminated 6 days postinoculation, with autopsy to view and score intestinal lesions and weight gains.

III. Vaccination and Immunization studies.

Vaccination of Pheasants: using *E. phasiani*. On the basis of improved clinical signs and growth, all four vaccinated groups had excellent immunity to reinfection.

Vaccination of Chukars:

1. Results suggested that chukars in all groups were highly immune to reinfection. Surprisingly, there appeared to be cross-protection between the two species of coccidia used in this study.

2. Using the experimental vaccine (*E. kofoidi* and *E. legionensis*), in comparison with a commercial vaccine (Coccivac-T, 1x or 10x) containing *E. dispersa*: several weeks post vaccination all groups were highly resistant to challenge with virulent chukar coccidia (*E. kofoidi* and *E. legionensis*).

3. Several weeks after vaccination, birds were challenged with virulent coccidia. Results showed that the vaccinated groups were all highly resistant to infection. However, examination of the data indicated that infection had spread from one treatment to the other, so that the chukar coccidia had spread throughout the birds of the test. This resulted in extensive mortality in all pens. Overall, mortality totaled 14-22% in the various treatments. This suggests that chukar coccidia are too virulent and contagious to be used as a vaccine without attenuation. Further, the test could not be properly evaluated, because the immune protection of Coccivac-T birds could be due to contamination by chukar coccidia from the other treatment. Further tests are underway to answer this question. It can also be noted that after suffering coccidiosis, birds in all treatments grew normally and were healthy when the test was terminated at 8 weeks of age.

Vaccination of Bobwhite Quail:

1. Clinical signs were greatly reduced and reproduction of the coccidia almost completely eliminated by both levels of vaccination.

Discussion

Coccidia were isolated from samples obtained from outbreaks on farms in 22 states. A total of 30 isolates were from pheasants, 20 from chukars and 30 from bobwhite quail flocks. Using a combination of direct microscopic examination, biological properties and molecular techniques (PCR), we identified *Eimeria colchici* and *E. phasiani* as the most pathogenic species in pheasants, *E. kofoidi* and *E. legionensis* in the chukar, and *E. lettyae* in bobwhite quail. These species were always present in outbreaks involving extensive mortality. Other species were also present in pheasants and quail, but were of lesser pathogenicity. One or more unidentified new species were observed in chukars and quail, but were rare.

Various anticoccidial products available for use in other poultry were tested for effectiveness in preventing coccidiosis in these birds. The products varied widely in effectiveness against the modern isolates. Some older products, such as amprolium, were not effective even at high levels, presumably because of build-up of drug resistance in the coccidia because of many years of usage. Some drugs had little innate effectiveness, as has been previously reported in the literature. Lasalocid (Avatec), the most widely used product in pheasants and chukars, had moderate to

good activity, although much less than reported in the 1980s. Other ionophores (related drugs) were not as effective as Avatec. Some synthetic chemical products had excellent activity. Robenz, Deccox, and Clinacox were highly effective. However, it is cautioned that the use of these products should be limited, as the risk of rapid drug resistance is high. This was confirmed by the isolation of highly resistant coccidia from a farm where Robenz had been used earlier in the season. The isolate was also resistant to most other products as well. Rofenaid, a potentiated sulfa product, was highly effective against most isolates, but probably should be reserved for treatment rather than prevention because of its cost. Continued testing during the second year of the study showed that there was considerable variation in the response of field coccidia to drugs, probably as a result of differing history of product usage in different locations.

During the second year (part III) of the study, we concentrated on establishing principles of immunization in the bird hosts. Studies were conducted with each species of bird to show that the birds would indeed become immune to reinfection after initial exposure, as has been shown for chickens and turkeys. Using small doses of live coccidia, we were able to induce protection in pheasants, chukars and bobwhite quail, which was effective against a severe exposure. Surprisingly, there seemed to be cross-protection between some of the types of coccidia. Further studies were done with birds on litter floors, simulating a commercial production environment. Chukars vaccinated with live coccidia and with Coccivac-T (a commercial turkey coccidiosis vaccine), developed protective immunity, but suffered significant mortality during the immunization process. This suggests that live chukar coccidia are too prolific to be used in this way. The vaccine strains would have to be attenuated for this approach to be practical. The results with Coccivac-T were promising, but further studies are needed to prove that the birds were actually becoming protected from the exposure or whether accidental contamination from the chukar coccidia was responsible. These results have prompted some to initiate field studies with Coccivac-T in chukars. Thus far the results of these tests are encouraging. If attenuated strains of coccidia are required for use in vaccines, additional funding will be required for their development.

References

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