The Effect of Quinacrine Hydrochloride on Cecal Coccidiosis of Chickens

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Coccidia of fowls were seen at various times during the later half of the nineteenth century. However, it was not until the works of Tyzzer, Theiler, Jones, and Johnson, published between 1923 and 1932, that a clear distinction was made between the species involved; that the life cycles were traced, and that the pathology was described.

Of the six commonly recognized species of coccidia infecting chickens, (namely, *Eimeria tenella*, *E. mitis*, *E. acervulina*, *E. maxima*, *E. necatrix*, and *E. praecox*,) *E. tenella* is undoubtedly the most highly pathogenic. Its life cycle and host-parasite relationships are essentially the same as those of the other five species. The life cycle, according to Becker (1934), is as follows: *Eimeria tenella* is an intracellular sporozoan parasite of the cecum of the chicken. The bird is infected by ingesting sporulated oocysts which excyst as a result of the action of pancreatic and duodenal enzymes upon them. The liberated sporozoites migrate into the cecum where they invade the epithelial cells of the mucosa. The sporozoites, now called schizonts, undergo nuclear and cytoplasmic division to give rise to the first generation merozoites. The mature merozoites break out of the epithelial cells and invade nearby epithelium. The infected epithelial cells become considerably enlarged and migrate to a sub-epithelial position where they serve as host to second generation merozoites. When the second generation merozoites are fully developed, they erupt from the epithelial cells. Extensive hemorrhage and mucosal sloughing occurring at about this time liberate the merozoites from their sub-epithelial position. The merozoites again penetrate epithelial cells wherever they may persist and become the precursors of gametocytes. The epithelium invaded by these second generation merozoites is not stimulated to growth nor does it develop the wandering properties of cells infected by first generation merozoites. Whether or not this process of asexual reproduction may continue indefinitely, or is limited, is a point which has not been positively settled. However, the consensus of opinion is that the infection is a self limiting one. After these two schizogonies, the parasites enlarge and assume the morphological characteristics of either macrogametocytes or microgametocytes which subsequently become macrogametes or microgametes. The mature gametes are squeezed out of the epithelial cell and fertilization, which has never been observed, probably ensues. The fertilized oocysts are voided by the bird and final development, the formation of the sporocysts and sporozoites, takes place in the soil.

In general, the species of *Eimeria* infecting chickens show a marked host specificity. All of these species induce immunity reactions. The

immunity to a single species is effective only against that species, for cross-immunity reactions do not occur.

Following the ingestion of sporulated oocysts, there is a seven day prepatent period at the end of which time oocysts may be demonstrated in the droppings. However, birds receiving lethal infestions may not live through the prepatent period but show symptoms of severe hemorrhage on the fourth or fifth day, the infection causing death on the fifth, sixth, or seventh day.

Methods of treatment involve the use of drugs; i. e., arsenicals, sulfa derivatives, sulphur, mercurials, and others; and modifications of diet. These remedies leave much to be desired. Some are highly toxic, resulting in retarded development of the host. Some have only prophylactic value, since they are not effective after symptoms appear. Prognosis in coccidial infections in chickens is not good.

The fact that quinacrine hydrochloride or "atebrine" has been shown to be very effective against malarial protozoa; the coccidium, *Isospora hominis*, Railliet and Lucet, 1901, parasitic in man; and, according to Brumpt (1942), treatment of coccidial infections of rabbits, led us to believe that it might be effective in cecal coccidiosis in chickens.

Dr. C. A. Herrick of Wisconsin University graciously supplied us with a culture of oocysts of *Eimeria tenella*, Railliet and Lucet, 1891. In order to build up the culture, sub-lethal doses of these were given to a group of birds. The resulting oocysts were sporulated in aerated, five percent aqueous potassium dichromate. Sporulated oocysts in normal saline stored in the refrigerator survived better than non-sporulated oocysts. The crystalline quinacrine hydrochloride used in the experiments was generously given by the Department of Medical Research, the Winthrop Laboratories, New York. Only freshly made solutions of quinacrine were given to the chickens as solutions of the drug tend to become toxic upon standing.

Fifty-six of the ninety chickens used in our experiments were of the Brown Leghorn variety and were hatched in the laboratory. Thirty-four White Rocks were obtained from the Farm Bureau hatchery as one-day-old chicks. Every precaution was taken to prevent accidental infection of the stock colony. The young chicks were kept in a heated, wire-floored brooder battery, and older birds were kept in wire-floored cages. The brooder and cages were cleaned daily. Chicks up to twelve weeks old were give a dry, milk-sugar starter diet, and older birds were fed a fifty-fifty mixture of chicken scratch and cracked corn. Food and water were kept in the brooder and cages at all times.

Three different parasite-drug combinations were used to test the effectiveness of the quinacrine. In the first experiment, fifteen, twelve-week-old Brown Leghorn chickens were banded and divided into three groups of five birds each. Each group was placed in a large wire-floored cage and the cages were widely separated. The birds in group one served as the drug control group, receiving quinacrine but no coccidia, and will hereafter be referred to as the quinacrine control group. Group two was the drug test group. The birds in this group were given both coccidia and quinacrine. Hereafter, the birds in this group will be re-

ferred to as the quinacrine test group. Group three functioned as the coccidium control group and received only parasites. The initial infections of the quinacrine test group and the coccidium control group were accomplished by giving the chickens, orally, by pipette, approximately 20,000 sporulated oocysts of *Eimeria tenella*, Railliet and Lucet, 1891. Twenty-four hours after this initial infection three birds were given a booster infection of 20,000 sporulated oocysts. Immediately following the administration of the second dosage of coccidia to the quinacrine test group and the coccidium control group, drug therapy was started on the birds in the quinacrine control and quinacrine test groups. The drug, dissolved in distilled water, was administered orally, by pipette, and each bird received ten milligrams of quinacrine per kilogram of body weight every four hours, five times a day, for seven days. Thus each bird received, in toto, 350 milligrams of drug per kilo. Droppings were checked daily for parasites. Seven days after the initial infection, a few oocysts were found in the feces of the chickens in the quinacrine test and the coccidium control groups. Concentrations of the droppings of the quinacrine control group failed to reveal any parasites. Drug therapy was suspended for twenty-four hours, and the birds in the quinacrine test and coccidium control groups were re-parasitized with approximately 300,000 sporulated oocysts of *E. tenella*, per bird, given in two 150,000 oocyst infections, twelve hours apart. Quinacrine therapy was resumed in the quinacrine control and quinacrine test groups, each bird receiving fifty milligrams per kilo of body weight every twenty-four hours for seven days. Seven days after reinfection the birds showed symptoms of coccidiosis and oocysts were present in the droppings. Concentrations of droppings from the quinacrine control group revealed no parasites. All droppings in the quinacrine test group and the coccidium control group, containing occysts, were carefully transferred to two five per cent solutions of potassium dichromate. After forty-eight hours in the dichromate solution all fertilized oocysts, comprising ninety-five per cent of the total number, were sporulated. In making the oocyst counts, the droppings were first washed with tap water to remove the larger food particles. After forty-eight hours the oocysts settled out completely and the excess fluid was drawn off. Both specimens were diluted to 500 cc. with normal saline. In making the counts, the specimens were agitated thoroughly to insure uniform distribution of the oocysts. A sample of the specimen was quickly pipetted off and placed in a haemacytometer chamber, and the number of oocysts in 0.9 cubic millimeters of sample was determined. At least five counts were averaged for each specimen.

In the second experiment, fourteen thirteen-week-old Brown Leghorn chickens were divided into two groups of five birds each, and one group containing four birds, as in the first experiment. The quinacrine test group and the coccidium control group received approximately 300,000 sporulated oocysts of *E. tenella* as a 150,000 oocyst initial infection and a 150,000 oocyst booster given six hours after the first infection. The purpose of this experiment was to determine the value of quinacrine as a therapeutic agent when administered after symptoms appear. Seven days after the initial infection, the birds in the quinacrine test group

and the coccidium control group showed symptoms of coccidiosis; that is, blood, mucus and occasional trophozoites in the droppings, leg weakness, and decreased food and water intake. At this time drug therapy was started on the birds in the quinacrine control group and in the quinacrine test group. These birds received orally, by pipette, fifty milligrams of quinacrine per kilo of body weight twice a day. The dosages were given twelve hours apart. Drug therapy was continued for two days so that each bird received 200 milligrams of quinacrine per kilo of body weight. Droppings, containing oocysts, from the quinacrine test group and the coccidium control group were kept separate and treated as in Experiment One. The quinacrine control group was negative for accidental infection.

In Experiment One, infected birds receiving no quinacrine had an average oocyst count of 34 per 0.9 cubic millimeter sample of droppings, while infected birds receiving quinacrine had an average oocyst count of 32 per 0.9 cubic millimeter. In Experiment Two, infected birds receiving no quinacrine had an average oocyst count of 232 per 0.9 cubic millimeter sample of droppings, and infected birds receiving quinacrine had an average oocyst count of seventy-seven per 0.9 cubic millimeter. Although the birds in Experiment One received somewhat heavier infections, their oocyst counts were considerably less than the counts in Experiment Two. This difference in oocyst count possibly can be attributed to the manner in which the birds were infected. The birds in Experiment One were given two infections of oocysts with sufficient time between them for the first to have been completed before the second infection was given. Hence, there is a possibility that a partial immunity was developed. The difference between the oocyst counts in Experiment Two apparently can be attributed to the action of the quinacrine in reducing the production of oocysts.

Experiment Three was designed to determine the therapeutic value of quinacrine in lethal coccidial infections. In this experiment, twenty three-week-old White Rock chicks were placed in individual wire-floored cages, and the cages were divided into four equal groups. One group of birds received neither coccidia nor quinacrine. The quinacrine control group was given only the drug. The quinacrine test group received both quinacrine and a lethal infection of coccidia, and the coccidium control group received only a lethal infection of oocysts. An initial infection of approximately 200,000 sporulated oocysts of *E. tenella* was given each chick in the quinacrine test group and in the coccidium control group. A booster dose of approximately 200,000 sporulated oocysts was given each bird forty-eight hours after the first infection. The quinacrine therapy, which was started twenty-four hours after the initial oocyst infection, was given orally, by pipette. Each bird in the quinacrine control group and the quinacrine test group received one fifty milligram dose of quinacrine per kilo of body weight every twenty-four hours. Therapy was given for five days; thus, each bird received a total of 250 milligrams of quinacrine per kilo of body weight. All infected birds showed symptoms of the disease five days after the initial infection, and all died on the sixth day. Controls were negative for infection. The conclusion drawn from

this experiment is that quinacrine hydrochloride in a dosage of fifty milligrams per kilo of body weight per day does not have therapeutic value in cecal coccidiosis in three-week-old chickens given lethal infections of *E. tenella.*

Dosages of quinacrine up to fifty milligrams per kilo of body weight per day, for a maximum of fifteen days, had no apparent injurious effect upon the chickens.

Measurements of oocyst size and pathological findings indicated that we were using *E. tenella.*

Summary

1. Sublethal doses of *Eimeria tenella,* Railliet and Lucet, 1891, seem to produce prompt immunity.

2. Quinacrine hydrochloride may reduce the number of oocysts voided in the feces in sublethal infections of *E. tenella.*

3. Quinacrine hydrochloride in a dosage of fifty milligrams, per kilo of body weight, per day, does not have therapeutic value in cecal coccidiosis in three-week-old chickens given a lethal infection of *E. tenella.*

4. Dosages of quinacrine hydrochloride up to fifty milligrams per kilo of body weight per day, for a maximum of fifteen days, had no apparent injurious effect upon the chickens.

Literature Cited

Becker, Elery R., 1934. Coccidia and Coccidiosis of Domesticated, Game and Laboratory Animals and of Man. Collegiate Press, Inc., Ames, Iowa.

Brumpt, L. C., 1942. Le Traitement des Coccidiosis des Animaux Domestiques Par la Quinacrine au Atebrine. Ann. Parasitol. Humane et Coup, **19**:97-115.

Johnson, W. T., 1923. Avian Coccidiosis. Poultry Sci., **2**:146-163.

——— 1927. Immunity or Resistance of the Chicken to Coccidial Infection. Ore. Agr. Exper. Sta. Bull. No. 230.

——— 1927a. Two Basic Factors in Coccidial Infection of the Chicken. Jour. Am. Vet. Med. Assn., **70**:560-582.

——— 1928. Coccidiosis of the Chicken. Ore. Agr. Exper. Sta. Bull. No. 238.

Jones, E. E., 1932. Size as a Species Characteristic in Coccidia: Variations under Diverse Conditions of Infection . Arch. f. Prot., **76**:130-170.

Tyzzer, E. E., 1927. Species and Strains of Coccidia in Poultry. Jour. Parasit., **13**:215.

——— 1929. Coccidiosis in Gallinaceous Birds. Am. Jour. Hyg., **10**:269-383.

——— 1932. Criteria and Methods in the Investigation of Avian Coccidiosis. Science, **75**:324-328.

Tyzzer, E. E., Theiler, H., and Jones, E. E., 1932. Coccidiosis in Gallinaceous Birds. II. A Comparative Study of Species of *Eimeria* of the Chicken. Am. Jour. Hyg., **15**:319-393.