SOME ASPECTS ON CHICK MORTALITY WITH PURE AND MIXED INFECTIONS OF THE PROTOZOAN PARASITES, EIMERIA TENELLA AND EIMERIA NECATRIX AND IN VITRO STUDIES ON THE EFFECT OF AUREOMYCIN ON THE SPOROZOITES AND MEROZOITES OF E. TENELLA

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
William Dunsmore Wilson
1957

This is to certify that the

thesis entitled

Some Aspects on Chick Mortality with Pure and Mixed Infections of the Protozoan Parasites, Eimeria tenella and Eimeria necatrix and In Vitro Studies on the Effect of Aureomycin on the Sporozoites and Merozoites of E. tenella.

presented by

William Dunsmore Wilson

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Microbiology

William D. Lindquist
Major professor

Date May 8, 1957

O-169

SOME ASPECTS ON CHICK MORTALITY WITH PURE AND MIXED INFECTIONS OF THE PROTOZOAN PARASITES, *EIMERIA TENELLA* AND *EIMERIA NECATRIX* AND *IN VITRO* STUDIES ON THE EFFECT OF AUREOMYCIN ON THE SPOROZOITES AND MEROZOITES OF *E. TENELLA*

By

William Dunsmore Wilson

A THESIS

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

1957

ACKNOWLEDGMENTS

To:

Dr. William D. Lindquist, for the guidance and advice that made this work possible;

Dr. Emanuel Waletzky, American Cyanamid Company, whose suggestions were most helpful and who generously supplied the Aureomycin;

Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, for making this work possible in the form of a Research Fellowship and by underwriting the cost of chicks and other expendable materials; and

Dr. William D. Baten, for his statistical assistance,

The author wishes to express his sincere appreciation.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

The magnitude of literature dealing with poultry coccidia would lead one to believe that these protozoan parasites have been "tamed." Indeed, certain phases (e.g., life cycles) have been determined in great detail. However, in other phases (e.g., mortality with respect to inocula) there is much to be desired. In some studies where mortality is used as an indication of resistance, immunity and drug effect it has been reported that a few thousand oocysts were capable of causing 50 percent mortality while in other experiments, one hundred thousand oocysts were reported to cause little or no mortality. Recognizing these inconsistencies, cecal lesions have been graded and weight gains and losses have been utilized in conjunction with mortality. However, these have not proved entirely satisfactory since uniform grading of lesions is difficult and weight gains, to be significant, require many weeks of chicken care and management if very young chicks are used.

Indagations of a correlation between age and mortality have been recorded, but although much work has been reported, this question remains unsettled.

Associated with mortality results, one must be aware of the fact that the two most pathogenic coccidia of chickens

(Eimeria tenella and E. necatrix) cannot be readily separated by size, sporulation time or location of oocysts. They can easily be distinguished from each other by pathological sequalae and by merozoite infections. Nevertheless, there are undoubtedly many cases of mixed infections reported as a single infection. What effects mixed infections would exercise on mortality is not known. Mixed infections have been reported in drug studies, but the controls consisted of a like mixed infection, which gives no indication of the effect of individual species.

Of the prodigious amount of literature on the prophylaxis and chemotherapy of poultry coccidiosis, the majority of conclusions have been based on mortality and weight gains which are difficult to confirm due to fluctuations of both criteria. Other methods (e.g., histopathology) have been employed as an aid in determining which, if any, phase of the life cycle is affected by a compound.

Originally a program was set up to investigate the influence of bacteria on coccidia and to determine what role Aureomycin HCl* might play in the overall picture. However, before one can investigate these questions others must first be answered.

Therefore, the problems to be studied which are unresolved are:

1. Can LD_{50} determinations be made for poultry coccidia?
2. If so, can these results be duplicated?

---

*Aureomycin® HCl = crystalline chlortetracycline HCl.

3. What influence, if any, does mixed infection play in mortality?
4. Does the administration of Aureomycin to test animals affect mortality?
5. If so, is this influence exerted by direct action on the parasite?

LITERATURE

Until Tyzzer's (1929) salient monograph, all coccidia of the fowl were known and studied as Eimeria avium, with few exceptions. Railliet and Lucet (1891) described Coccidium tenellum from the diseased ceca of chickens which Railliet (1913) later corrected to Eimeria tenella. Gerard (1913) described what he thought was a new species, Eimeria bracheti, which, from his description, was later considered to be E. tenella. Other workers of this period, Cole and Hadley (1908), Hadley (1909), Eckardt (1903), Fantham (1910, 1915), and Young (1929) indicated E. avium as the etiological agent of white diarrhea, roup, blackhead in fowls, fowl paralysis, and liver involvement, as well as being transmissible from grouse to chicken and from chicken to turkeys, pigeons, geese, ducks and other fowl. All of these have later been refuted by the original author himself or by others, such as Johnson (1923) and Tyzzer (op. cit.).

Isolation, developmental time, topographical distribution, associated gross pathological changes and symptoms, intestinal mucosa, morphology (including size and shape of oocysts), pathogenicity and the reaction of host-tissue were all taken into consideration by Tyzzer (op. cit.) in describing E. tenella and three new species: Eimeria mitis, Eimeria acervulina and Eimeria maxima. Thus, for the first time

morphological characteristics were established whereby new species of coccidia could be discovered and described. Due to the work of Tyzzer (op. cit.), Johnson (1930), Tyzzer, Theiler and Jones (1932), and Levine (1938, 1942a) we now recognize eight species of chicken coccidia, each of which pursues its own independent life cycle, as being responsible for coccidiosis of chickens. Of these eight, only two, E. tenella and Eimeria necatrix, Johnson (op. cit.) will be subsumed in this investigation.

Eimeria tenella, Railliet and Lucet (1891)

Life cycle:

Rivolta (1878) described white points the size of a poppy seed in the submucosa of the intestines of fowl and Schaudinn (1900), Fantham (1910), Hadley (1911), and Gerard (1913) gave general accounts of life history, in some cases figures of various stages of development. It was not until Tyzzer (1929) and later Scholtzseck (1953) that the life cycle and morphological characteristics of E. tenella were described in detail. In fact it was not until 1929 when one could be sure that one, and only one, organism was involved in any of the aforementioned descriptions. A brief résumé of the life cycle of this parasite is as follows: oocysts passed out of the chicken in the feces--sporulation with the development of four sporocysts which are made up of a total of eight sporozoites--fully sporulated oocysts eaten by the chicken--

sporozoites liberated in the small intestine--migrate to ceca--penetrate epithelium--schizont (first generation) formed in which develops merozoites (first generation)--merozoites liberated (an estimated 900 from one sporozoite) from the enlarged cell--lumen of ceca (2 1/2 to 3 days after oocysts eaten)--merozoites invade epithelium of the fundi of the glands--develop into schizonts (second generation)--merozoites, 200-300 from one first generation merozoite (second generation), develop--merozoites liberated five days after infection and penetrate glandular epithelium--a few may develop into third generation schizonts and merozoites, but the majority develop into micro- and macrogametocytes--micro- and macrogametes formed--microgametes liberated--penetrate macrogametes--oocysts liberated--pass out in the feces (total of seven days after ingestion of the sporulated oocysts). This prepatent period, time of oocyst ingestion to time of passage, may, under certain conditions, require only six days for this species and E. necatrix (Edgar, 1955).

Pathology:

Due to conflicting reports and misunderstanding of the etiological agents of chicken coccidiosis, it is small wonder that the true gross pathology and histopathology picture was non-existant until the work of Tyzzer (1929). Since white diarrhea, roup, blackhead, fowl paralysis and other diseases of fowl were attributed to coccidiosis, it was almost impossible to separate the pathological picture of

E. tenella from that caused by other species of coccidia and other etiological agents. Nonetheless, from descriptions of the parasite and the pathology involved, one is able to assume in some cases, e.g., Gerard (op. cit.), that one of the main etiological agents involved was E. tenella. However, the true pathological picture, in which the etiological agent of cecal coccidiosis was itself the instigator, was not described in detail until 1929 (Tyzzer), 1937 (Mayhew), and in 1953 (Greven). While histopathological events are taking place in the ceca of infected birds during the formation of the Generation I and II schizonts and merozoites, it is not until the Generation II merozoites are liberated that external symptoms of infected birds are observed. This usually occurs on the fifth day as large quantities (dependent upon the magnitude of the infection) of unclotted or partially clotted blood is passed in the feces. Presence of blood is due to the leakage and rupture of blood vessels as developmental stages reach maturity and are released into the lumen. Whereas gross external pathology cannot be observed until 5 days after infection, a microscopic study of the feces from 2 1/2 to 6 days after infection will reveal merozoites and on the seventh day, oocysts.

Gross pathological studies on the ceca of infected birds reveal observable hemorrhages, either as small pinpoint spots or a profuse area, dependent upon the severity of infection, on the fourth day after infection. This hemorrhaging increases

on the fifth day and then diminishes until approximately seven days after infection, and the ceca are mottled reddish or a milky white in color due to the loss of hemoglobin and the presence of a large number of oocysts. After this period, the ceca essentially return to normal. It should be noted, however, that the ceca are usually larger than they were previous to the infection and that the efficiency of the ceca may be impaired due to connective tissue which replaces cells which have been destroyed. It is possible for unusual gross changes to occur if a large core (formed by mixtures of destroyed tissue and clotting blood) is retained, or adhesions occur or if the ceca are ruptured.

See Appendix, Plate I.

Oocyst studies:

Henry (1932) found that the cyst wall of various species of Eimeria (E. tenella and E. necatrix excluded) contained up to three parts. Gill and Ray (1954b) reported a hyaluronic acid type polysaccharide (HAP) in the protective covering of the oocyst of E. tenella which ensures safe passage of oocysts through acidic medium of the proventriculus of birds. The latter authors also believe these mucopolysaccharides are of great value to the organism by inhibiting intra-cytoplasmic clotting, helping in multiplication and gamete formation and by protecting merozoites against coagulation. The sporocyst wall and oocyst wall (E. brunetti and E. acervulina contain some polysaccharide, either free and/or

combined and some protein. However, the so-called plastic granules which later make up a large part of the oocyst wall are composed largely of muco-protein (Pattillo and Becker, 1955). The actual structure of the oocyst wall is not known.

Warner (1933) found that birds did not become infected from soil which had been free of infected birds for 81-370 days, but that seeded soil gave positive results for 197 days. Intermittent freezing of oocysts in soil had no effect for at least 12 weeks and drying killed oocysts of E. tenella in four weeks. (Patterson, 1933). Dessication, putrefaction, lack of air and direct sunlight prevent sporulation and destroy oocysts rapidly (Benedetti, 1944). E. tenella oocysts will sporulate at 29° C at a relative humidity as low as 60 percent, although the outer wall is broken and spores are released (Brotherston, 1948). Warner (op. cit.) and Ellis (1938) reported that chicken eggs immersed in suspensions of sporulated oocysts no longer contained viable oocysts on the shell after normal incubation periods.

Farr and Wehr (1949) reported E. tenella as having disappeared from the soil of all test plots in less than a year and that oocysts of E. acervulina were recovered from the plots after 86 weeks. Koutz (1950) found that E. tenella oocysts were infective (both by feeding soil and under natural conditions) for 272 days exposure from September to June, but did not survive a severe winter and partial summer up to 322 days. In other tests the same author found that E. tenella

oocysts did not survive as long as did E. maxima, E. acervulina, and E. mitis. Delaplane and Stuart (1935) reported oocysts surviving in plot soil through the winter and spring for months after the removal of fowls from the plot. Soil of a wooded range showed viable oocysts at 15-18 months after removal of all fowl.

In studies with deep litter, Boughton (1939) found that excessively wet litter inhibited sporulation while a limited amount of moisture enhanced sporulation. Koutz (1953) in his work with deep litter found that the oocysts of E. tenella and other Eimeria remained viable during the one year the experiments were carried out. Tomhave (1949) stated that death losses were lower and chickens weighed more when raised on dry litter which contained oocysts, as opposed to chickens raised on wet litter containing oocysts. Baby chicks started out on re-used built up litter (compost litter) became heavily infected with coccidiosis and were not as heavy as those raised in batteries free from coccidia (Skoglund, 1952).

Methyl bromide appeared to be immediately effective against E. tenella oocysts in the soil (Clapham, 1950). Boney (1948) found that this same gas, when used as a fumigant would kill sporulated oocysts when used at the rate of one pound per one thousand square feet. In 1940, Horton-Smith, Taylor and Tuttle reported ammonia fumigant effective in destroying oocysts.

Fish (1931) reported that 5 percent colloidal iodine (Iodine Suspensoid, Merck) and 2-5 percent cresol produced 100 percent mortality of unsporulated oocysts in less than 24 hours and that ultraviolet light was also effective. However it is important to remember that in laboratory tests the oocysts have been relatively freed from most excess organic matter. Therefore, chemical or physical methods may be effective but under natural conditions may be quite ineffectual. One should also take cognizance of the fact that a compound must kill rapidly to be practical. Andrews (1933) found that oily mixtures of phenolics were effective against the oocysts of E. tenella. Using colloidal iodine, Chandler (1933) reported three gallons of suspension containing 0.2-0.4 percent iodine content per 100 square feet of surface were sufficient to accomplish practical disinfection providing the brooder floors were thoroughly cleaned. Chandler (op. cit.) also reports that to exert lethal action on oocysts, the colloidal iodine must be in contact with them, while the iodine is in the free state, from one to two minutes. Anderson and Mallmann (1945) reported that, of the compounds tested, colloidal iodine was the only compound which possessed marked powers of penetrability. Uricchio (1953) reported that allylacetone and dibromobutene (pure and in emulsions composed of 50 percent sorbitol esters of mixed fatty acids) prevented sporulation and killed sporulated oocysts.

Fish (op. cit.) found that the thermal death time of unsporulated oocysts is inversely proportional to the degree of heat used (24 hours at 45° C - 5 seconds at 80° C) and that moist heat at 55° C killed both sporulated and unsporulated oocysts. Perard (1925) reported the Eimeria perforans (host: rabbit) oocysts were unable to sporulate if kept in an aqueous medium for one day at 40° C and that 80 percent of the oocysts were killed within 20 minutes in water at 55° C, within 10 seconds at 80° C, and within 5 seconds in boiling water. The same author also presented evidence that dessication destroys oocysts completely, putrefaction was detrimental and that no sporulation occurred at 0° C and 2° C. In experiments with E. perforans and E. magna (host: rabbit) Becker and Crouch (1931) found that E. magna oocysts completed sporulation as follows: at 25° C - 50 percent sporulation within 84 hours, at 33° C - 80 percent sporulation within 72 hours; and that similar tests with E. perforans resulted as follows: at 25° C - 50 percent sporulation within 48 hours, and at 33° C - 100 percent within 48 hours. In 1954, Edgar found that E. tenella oocysts' maximum sporulation appeared to take place at 29° C for 22-24 hours and at 28° C for 27-30 hours. However, E. tenella and E. necatrix oocysts became infective in 18 hours (Edgar, 1955). Up until this report by Edgar, the sporulation time (time involved from oocysts passage until it became infective) was considered to be approximately 24-48 hours.

Pratt (1937) reported that he was unable to initiate excystation of oocysts by chemical means, however, Smetana (1933) and Goodrich (1944) were successful with the use of a trypsin solution.

Immunity:

Beach and Corl (1925) observed that chickens with previous infection with E. avium (=E. tenella) developed some resistance to reinfection. The first experimental evidence showing that immunity to coccidiosis may be acquired through previous infection was by Johnson (1927). While working with mixed species of Eimeria he likewise observed that a high degree of susceptibility was maintained in both developing and mature cage-reared fowl. Concerning this last observation, workers are not in agreement. There are those who maintain that the younger the birds the more susceptible they are, while others feel that very young birds are comparatively immune (Becker, 1952). Tyzzer, Theiler and Jones (1932) using E. necatrix observed that younger birds fed massive doses of oocysts were less severely affected than older birds. Brackett and Bliznick (1952), using the same pathogen, concluded that younger birds are more severely affected than older birds. Gordeuk et al. (1951) found that chicks as young as one day were susceptible to cecal coccidiosis and developed some immunity. In 1936, Herrick, Ott and Holmes reported chickens up to and including 15 months of age were susceptible to E. tenella infection, and that those three

months or older were more resistant than those younger than two months. Horton-Smith (1947) demonstrated that if chickens are kept free of infection, six month old animals are just as susceptible as younger birds. Gardiner (1955) using E. tenella found that chicks one to two weeks old were more resistant than older chicks and that the greatest susceptibility during the first six weeks of their life occurred at the age of four weeks. While the question of age and susceptibility still remains unresolved, other workers, Mayhew (1934), Herrick (1934), Rosenberg, Alicata and Palafox (1954) reported that hereditary resistance and susceptibility to cecal coccidiosis exists in chickens. Both Champion (1954) and Rosenberg, et al. (1954) reported that selective breeding was effective in establishing lines of chickens resistant and susceptible to cecal coccidiosis, that factors for resistance or susceptibility do not exhibit a strong order of dominance, that sex-linked, maternal effect or cytoplasmic inheritance do not play a significant role in resistance or susceptibility. Along this same line Rosenberg (1941) reported that the Barred Plymouth Rock and Jersey White Giant had higher mortality rates than did the White Leghorn, New Hampshire and Rhode Island Reds.

Johnson's (1927) was not only the first reported study on immunity but was the first to use graduated doses of sporulated oocysts to accomplish this end. Tyzzer (1929), Farr (1943), Jankiewicz (1942) and Babcock and Dickinson (1954) have all reported immunity studies with varying dosage levels.

The latter authors reported that the time for immunity to develop (chickens receiving a total dosage of 1,050) is 4 days longer than for those whose graduated dose totaled 2,125. These authors further indicated that the minimum time required for immunity (600 oocysts the first day and 1,000 the second day) was six days with a culture more than 300 days old and three days with a culture less than 150 days of age. Jankiewicz (1942) reports that immunity increases with increase of oocysts given from 50-3,000 and that from 6,000-100,000 the immunity is the same. Gordeuk et al. (1951) indicated that in addition to age and degree of exposure other unknown factors are included in the immunity process.

Using compost litter to rear chickens for the purpose of developing immunity is at best unreliable (Koutz, 1955).

That reinfection may occur was pointed out by Tyzzer (1929), Tyzzer, Theiler and Jones (1932), Farr (1943) and Waletsky and Hughes (1949). Herrick (1935) showed, upon subsequent reinfection, no observable effect on chickens one year after immunity developed.

Waletsky and Hughes (op. cit.) point out that in laboratory studies on immunity it would be advisable to use more than a two week interval between primary and challenge inoculum due to duration and incidence of pathological sequalae which may give erroneous results in acquired immunity studies.

Many authors, among which are Allen and Farr (1943), Horton-Smith and Taylor (1945), Waletsky and Hughes (1946),

Seeger (1946), Swales (1946), Koutz (1948), Thorp, et al. (1947), Goldsby and Eveleth (1950), and Cuckler and Malanga (1955) have reported a substantial degree of acquired immunity following infection and using various medicated diets simultaneously. Using similar procedures, a practical vaccine was announced by Edgar (1956).

Some attempts have been made using attenuated oocysts in producing immunity in chickens. Jankiewicz and Scofield (1934) used heated oocysts. Waxler (1941a) used x-ray treated oocysts and Uricchio (1953) used oocysts altered by ultrasonics, radium, freezing and heat. The latter author found that the most effective degree of immunity produced was by feeding fifteen day old birds 100,000 oocysts exposed to -5° C for five days while the former two reported some success with heat and x-ray treated oocysts. A point raised by Babcock and Dickinson (1954) that, in the works of Jankiewicz and Scofield (op. cit.) and Waxler (op. cit.), it was doubtful whether or not attenuation occurred since the results obtained might have been due to the death of some of the oocysts, thus reducing the number of viable oocysts in the dose. This might also be raised in the case of Uricchio (op. cit.).

Attempts by Tyzzer (1929), Tyzzer, Theiler and Jones (1932) and Goldsby and Eveleth (1950) to demonstrate humoral antibodies yielded inconclusive results. Tyzzer in attempting to immunize chickens against E. tenella was unsuccessful using serum from immune birds, oocysts and merozoites injected in

order as above, subcutaneous and intraperitoneal, parenteral, and intravenous. Precipitin tests using dried and ground oocysts as the antigen were unsuccessful as reported by Tyzzer et al. Goldsby and Eveleth (op. cit.) reported that using cecal lining and contents of birds in the acute state of infection as the vaccine failed to produce immunity. In 1954, McDermott and Stauber demonstrated agglutinins for the first time in the sera of experimental birds. These agglutinins persisted for at least thirty days after infection and had maximum serum titers reaching 1-320 between the tenth and fifteenth days post infection. The antigen used by these authors was a suspension of merozoites.

Becker (1934) indicated that immunity may not be merely a depletion of available epithelial cells and also that if it is purely a local defense reaction, the immune principle is capable of spreading from cell to cell. Andrews (1934) reported that chickens developing non-fatal coccidial infections either (1) develop an immunity, or (2) the infection becomes patently chronic with oocysts discharging indefinitely and birds susceptible to superimposed reinfection.

Physiology, metabolism and digestion:

In 1934a, Mayhew found that normal birds, with ceca removed, attained normal weights in a short time as compared with those recovering from coccidiosis and that layers laid a normal number of eggs. Various authors agree that the ceca of chickens are not essential structures and are concerned

with absorptive and cellulose digestion processes (Dukes, 1955). Collier and Swales (1948) found that two-thirds of the metabolic activity of cecal tissue resides in the mucosa and that cecal tissue parasitized with E. tenella showed no increase of respiration from the normal but rather a slight decrease in active parasite stages. Smith and Herrick (1944) presented evidence that parasitized (E. tenella) tissue gave a marked increase in O_2 consumption of the epithelial cells. In 1949, Ripson et al. studied three areas of the ceca (neck, middle and tip) and found that in uninfected birds there was a significant difference in the QO_2 of these three areas. They also found that after infection these QO_2 areas were more uniform and that upon reinfection, the coccidia were more evenly distributed in these three areas.

That coccidia causes some effect on the temperature regulation mechanism was shown by Herrick (1950). He found that uninfected birds, when subjected to cold, increased their metabolic rate and maintained their body temperature within one degree of normal and that with infected birds, of like age and size, the metabolic rate decreased and the body temperature of some dropped as much as twenty degrees centigrade.

In 1954, Levine and Herrick reported that chickens infected with E. tenella were so weakened that they could only do one-half as much work (over a three-minute test period) as could uninfected birds. These same authors (1955) found that this loss of ability to do muscular work was more

pronounced in infected Single-Comb White Leghorns than Barred Plymouth Rocks, and that while the leg muscles of these birds contained the same amount of actomyosin per gram, the ratio of actin to myosin was 1:24 for the Leghorns and 1:8 for the B. P. Rocks.

Glycogen studies of E. tenella, Edgar et al. (1944) and Gill and Ray (1954a), have resulted in showing the presence of this polysaccharide in developing macro- and microgametocytes, macro- and microgametes, freshly passed oocysts, aged oocysts that failed to sporulate, and excysted sporozoites. Gill and Ray (op. cit.) indicate that the parasite metabolizes its glycogen from the host's blood sugar, possibly through the activity of akaline phosphatase. Ray and Gill (1954) also reported that the heavily infected zone of the ceca was devoid of akaline phosphate activity but that activity of this enzyme was demonstrated in various stages of the parasite. In 1950, Daugherty and in 1952, Daugherty and Herrick reported that material taken from the infected cecum inhibited the phosphorylation process and that the possible effects of cecal coccidiosis may be mediated through carbohydrate metabolism interferences. The distribution of acid phosphatases and 5- nucleotidase and their probable role in protein and polysaccharide metabolism was reported by Gill and Ray (1954c). Pratt (1940) reported that severe coccidiosis (E. tenella) caused an increase in blood sugar which was probably due to cecal hemorrhages since a similar increase could be initiated by

artificial bleeding. In 1941, this same author reported changes in liver and muscle glycogen levels during infection and advanced the hypothesis that by some mechanism, the additional blood sugar had come from material (probably lactic acid) from the muscle when body fluids were taken in to replace that lost from cecal pouches during the acute stage of infection. Herrick (1950) reported liver and muscle glycogen were lowered on the fourth or fifth day and that feeding sugar solution increased the blood sugar but not the stored glycogen as is seen with uninfected birds. Feeding concentrated saline to chickens during the hemorrhagic phase will permit the rise in the blood sugar to be maintained at a lower level (Waxler, 1941b).

Pattillo and Becker (1955) in their study with Eimeria brunetti and Eimeria acervulina of the chicken report the distribution of glycogen, free aldehydes, protein-carbohydrate complex, lipids, desoxyribonucleic (DNA), and ribonucleic (RNA) acid in the life cycle stages of these parasites. The distribution of DNA and RNA in various endogenous stages of E. tenella have also been reported (Ray and Gill, 1955).

In 1933, Herrick reported that the total nitrogen, non-protein nitrogen and hemoglobin of the blood was reduced during E. tenella infection.

Evidence is presented by Todd and Hansen (1948) that induced mild hyperthyrosis (thyroprotein thyroxine in diet) resulted in an increase of the mean oocyst count per gram

of feces. Gill (1955) presented evidence that in infected birds (300 oocysts per bird) induced hyperthyroidism stepped up the rate of production four-fold whereas induced hypothyroidism slightly lowered oocyst production as compared with the controls. Similar results were reported by Todd et al. (1949). Wheeler et al. (1948) reported thiouracil did not alter mortality or loss of body weight as compared with infected controls, but that thyroprotein showed more benefits.

Natt and Herrick (1955, 1956) report the use of the hematocrit as a quick and easy method for determining the severity of hemorrhage in coccidial infection and that chickens who lost 29 and 48 percent of their blood volume in ten successive bleedings required the same amount of time for the erythrocyte count to return to normal as those with cecal coccidiosis. They further report decreases in body weight, percent corpuscular volume and blood volume, but no change in plasma volume during the hemorrhagic phase of cecal coccidiosis.

Schildt and Herrick (1955) found that in most infected birds the feed was retained in the crop during the fifth day post infection and if not retained food passage was delayed while no change was noted with respect to food passage in the gizzard and small intestine. They also reported crop activity decreased on the third day after infection and that this activity ceased on the fifth day in most cases and that two weeks were required before the crop activity returned to normal.

Jones (1934) reported that chickens maintained on high protein diets maintained more normal weights, developed

immunity slower, and produced more oocysts than did birds on normal diet, although Allen (1932) found that this same diet caused infected birds to be slower in gaining back weights apparently due to chronic coccidiosis. Vitamin A at various levels and in dietary substances failed to produce any noticeable effect of resistance to coccidiosis (Wickware, 1949, and Jones, op. cit.). Vitamin K afforded some control of hemorrhage in cecal coccidiosis (Baldwin et al., 1941), but Hawkins (1945) reported the exact opposite. Kennard and Chamberlin (1949) reported that chicks on old built-up litter made the best rate of growth with lowest mortality with the complete or incomplete (all plant) diet. Becker and Waters (1938) found that combinations of large amounts of dried skim milk or dried buttermilk and wheat middlings in the ration predisposed chickens unfavorably to cecal coccidiosis attacks. While E. R. Becker did much work in this area with coccidia of the rat, Eimeria neischultzi, the exiguity of literature dealing with diet in E. tenella and E. necatrix infections is most pronounced. Delaplane (1953) stated there is no clearly recognized relationship between poultry nutrition and coccidiosis.

With respect to another possible predisposing factor in cecal coccidiosis, Riedel (1950) reported that weight and morbidity records indicate ascarid infected birds were less resistant to coccidiosis than controls.

Eimeria necatrix, Johnson (1930)

General:

Johnson (1930) and Tyzzer et al. (1932) independently discovered Eimeria necatrix and since the former's work was the first published, to him goes the credit for describing this species. However, it is worth mentioning that both of the above used Tyzzer's (1929) criteria for species differentiation and were in agreement in their descriptions, but Tyzzer et al. (op. cit.) contained more detail.

Life cycle:

The stages in this parasite's life cycle are the same as those present in E. tenella, the difference being in the type of cells parasitized. Sporozoites liberated from ingested sporulated oocysts penetrate the epithelial cells of the small intestine and develop first generation schizonts and merozoites. These merozoites invade the adjacent gland epithelium and develop into the second generation schizonts and merozoites, the latter when liberated proceed to the ceca where they invade the surface epithelium and develop into the sexual stages. As with E. tenella, the oocysts are passed in the feces in seven days.

E. necatrix infections can be initiated by introducing its Generation I merozoites into the crop or the intestine of chickens. On the other hand, merozoites of E. tenella are able to cause infection only when injected into the intestine

or cloaca, Levine (1940). Tyzzer (op. cit.) was unable to initiate E. tenella infection with cloacal injection of merozoites.

Pathology:

In acute infections, hemorrhages of the small intestine occur on about the fifth day post infection as a result of the growth and liberation of the second generation merozoites. As with E. tenella, merozoites of this species may be seen in the feces before the blood is observed.

Gross pathological studies of the small intestine in the earlier stages, toward the end of the fourth day, will show whitish opacities (schizonts), especially in the middle one third of small intestine, through the serous and muscular coats, but not visible through the mucous surface. On the fifth day these opacities are larger (approximately one millimeter in diameter) and the small intestine shows a pronounced swelling, various degrees of distension, a dull reddish hue and punctate hemorrhages. On the seventh day, the intestine appears pale and the white opacities are not so readily distinguished.

In cases recovering from an acute attack, there may be badly damaged intestine which will show scar tissue having replaced destroyed glandular tissue, the result of which may impair the efficiency of the digestive processes of the small intestine.

See Appendix, Plate II.

While E. necatrix infection is readily distinguished from E. tenella on the basis of gross pathology features, its sporozoites, merozoites and oocysts have morphological characters which separate each from the other, Tyzzer et al. (1932).

Oocysts:

Oocysts sizes range from 22.7 x 18.3 μ maximum to 13.2 x 11.3 μ minimum with 16.7 x 14.2 μ mean, Tyzzer et al. (op. cit); 24.4 x 17.2 μ mean Edgar (1955); and a 19.7 ± 1.82 μ x 16.7 ± 1.2 μ mean with a length range of 12.1 -28.9 μ x and a width range of 10.8 - 23 μ Becker et al. (1956).

Immunity, physiology, metabolism and digestion:

Chickens recovering from a severe infection of E. necatrix are clinically protected against reinfection, however, a very light infection results in slight protection, Tyzzer et al. (op. cit.). As with E. tenella authors are not in agreement concerning the relationship between age and susceptibility of the chicken. In one instance young birds (8 days old) were found to be less susceptible than older (35 days old) birds, Tyzzer et al. (op. cit.) and vis-a-vis evidence was presented that show younger birds (9 days old) to be more susceptible than birds 10 to 12 weeks of age, Brackett and Bliznick (1952).

While there is a prodigious amount of literature available with respect to E. tenella, the paucity of that concerning E. necatrix is conspicuous.

MATERIALS AND METHODS

A. Pure and Mixed Infection Studies

1. Source of Chickens

Chickens (White Rock - Vantress Cross - straight run) were obtained from Hess's St. Louis Hatchery, St. Louis, Michigan. The majority of these birds were sent to Michigan State University via parcel post and arrived at the East Lansing postoffice, where they were promptly picked up the same day they were hatched. Occasionally, although handled in a similar manner, chicks were sent through the University mail and did not reach this area until they were one day old.

2. Distribution of Chickens

Upon their arrival, the chicks were immediately taken to the animal disease barn and placed in a brooder, which was in an isolated room.

When the birds attained the desired age for the experiments, (ranging in age from 13-18 days, the majority being 14 and 15 days old), they were removed from the brooder and placed in large paper bags (thirty chicks per bag). They were taken to their respective isolated rooms and distributed in groups of ten, one group for each of the three levels in each brooder.

3. Care and Management

During the first part of the experiment, Room 2 was used to maintain stock birds and controls. Later this room was used for experiments with Aureomycin and the stock birds were raised in Room 1. Feed (in 50 lb. bags) was always maintained in Room 1 and removed to the other rooms as needed.

Upon entering the entrance room, street clothes were removed. At the door leading into the hallway, street shoes were removed and knee boots, maintained in the hallway, were put on. These boots were worn only in the hallway. The first room entered, on all occasions, was that containing the stock birds. These chickens were fed and watered before proceeding to the other rooms. The procedure for entering all rooms was as follows: hallway boots were removed at the doorway into the wash room, hands were washed and since no boots were maintained in these rooms this area was traversed in stockinged feet. Upon opening the door into the experimental room, boots, which were maintained in and never removed from this room, were put on.

Clean coveralls were taken into rooms which were used for experiments on the same day as the birds and inoculating material. These coveralls were put on when entering this room and worn until leaving the room. Coveralls were removed from the room with the fecal can when the "run" was terminated.

Chicks, when received, were taken to the stock room (1). Here they were placed in the five level brooder (never

more than 70 birds per one level). The temperature of the brooder levels used for rearing younger birds was maintained at approximately 33° C. The temperature of the lowest level, used for older control birds, was approximately 25° C.

Feed and water was supplied ad libitum, except for a three-hour period prior to infection. When the birds reached the desired age they were taken off feed and water for three hours. At the end of this period they were placed in large paper bags (thirty birds per bag) and removed to the desired rooms along with coveralls and inocula vials. Birds were removed and inoculated one by one and placed in the brooder. After inoculation feed and water trays were filled and chicks fed and watered ad libitum until they were sacrificed. These brooders were maintained at approximately 25° C and their rooms were maintained at a temperature which varied from 22° C to 28° C. Exceptions will be noted later.

All birds were fed and watered every day. When birds died, a necropsy was performed to ascertain whether or not coccidia could have caused their death. At the end of the "run," birds were sacrificed and the oocysts collected as mentioned under collection of oocysts. Dead birds were placed in the fecal cans as was the fecal material from the fecal trays and the remaining feed. The brooder, except for the frame, was disassembled, thoroughly cleaned, washed and re-assembled. The floor and walls were likewise cleaned. The fecal can and coveralls were removed and taken out of the

building via the door opposite the entrance to the hall. The coveralls were taken to a container and later cleaned. The fecal cans were taken to the incinerator and the contents burned. Using a hood, which contained one water and one steam jet, the cans were cleaned and taken to a deep sink, immersed in boiling water for thirty minutes, dried, and returned to their respective rooms.

Upon the completion of all the "runs" (E. tenella, E. necatrix, mixed infections and drug test) the floors of the hallway and wash rooms, as well as the entrance room, were washed with a 2 percent colloidal iodine solution. This solution was then permitted to dry on the floor.

To determine if accidental coccidial infection had occurred, the following procedures were followed. When the birds became seven days old and every other day thereafter, until the sixth day post-infection, composite fecal samples were collected and examined for oocysts. The sugar flotation concentration method (Morgan and Hawkins, 1953) was used as follows: the fecal material was mixed with water, allowed to stand for one-half hour, mixed, strained through two layers of cheesecloth, and poured into two 100 ml. round bottom centrifuge tubes (approximately one-quarter full). Saturated sugar solution was added until the tubes were three-quarters full. The suspension was thoroughly mixed and more sugar solution was added until the tubes were filled. The tubes were then centrifuged for one minute at approximately 1000 rpm.

Using a headed glass rod, the material from the surface film of the sugar solution was transferred to a glass slide and covered with a cover slip. An examination of this material was made, using a compound microscope (100X), for the presence of oocysts.

4. Source of Oocysts

Oocysts of both species studied (E. tenella and E. necatrix) were obtained from the Stamford Laboratories of the American Cyanamid Company. Their origin and data are as follows: E. tenella originating from Beltsville, Maryland; 103 previous passages; last harvest, prior to arrival (April 23, 1956) at Michigan State University, was on March 28, 1956. E. necatrix originating from California (some doubt exists as to this origin); 28 previous passages, last harvest, prior to arrival (as above), was on March 3, 1956. The preservation medium, in both cases, was a two percent potassium dichromate solution.

5. Single Oocyst Isolation

A piece of soft glass tubing (inside and outside diameters, three and five millimeters, respectively) was heated and drawn out into a fine capillary. By breaking this capillary at the approximate point of smallest diameter, two capillary pipettes were obtained. To ascertain if a capillary was open, rubber tubing with a mouth piece (type used with a Thoma red blood cell pipette) was attached and the mouth piece

placed in the mouth. The capillary pipette tip was immersed in a drop of water on a glass slide, which had been placed on the stage of a stereoscopic microscope. Air was then blown through the pipette. Whether or not air was passing through was determined by looking for air bubbles through the lenses of the microscope (magnification = 20x).

The oocyst culture was diluted until a drop, when placed on a glass slide and observed with a compound microscope (100x) revealed one or no oocyst per microscope field. The mouthpiece of the rubber tubing was placed in position, the capillary pipette tip was placed in tap water and allowed to fill by capillary action. After removal from this water, the tip was immersed in the drop of diluted culture on the slide and moved into the observable field of the microscope. A very small amount of water was blown out of the capillary and held out by applying the tip of the tongue to the mouth piece. By gentle manipulation, the tip of the pipette was brought up to an oocyst. When the tongue was released, capillary action drew the oocyst into the capillary. The pipette was then removed from the water and the water and oocyst in the capillary blown out onto a clean slide and checked under a compound microscope (as above) to determine if only one oocyst was present. This oocyst was repipetted by a similar pipette, which was neither placed in the drop containing the oocysts nor into the water used to fill the former pipette.

The material from the latter pipette was blown into a number five gelatin capsule. This capsule was placed in the esophageal-pharyngeal region of a bird, two weeks old, and blown into the crop using a short piece of flamed glass tubing whose inside diameter was slightly less than that of the capsule.

Chicks two weeks old were brought into the laboratory and infected as above. Immediately after the single oocyst was given to each bird, they were taken to their respective isolated room in the animal disease barn. Here they were placed in the brooder and remained there for six days. On the sixth day, the birds were brought into the laboratory to prevent oocysts from more than one bird contaminating the brooder and room, and each bird individually isolated. On the seventh day post-infection, the birds were sacrificed (one at a time) and the ceca were removed with forceps, washed in running tap water and placed in sterile petri dishes, which were numbered in the order of ceca removal. In order to avoid contamination from one bird to another, a sterile pair of scissors and forceps were used for each pair of ceca removed. Birds carcasses were destroyed. The material from the ceca in each petri dish was examined (in the same order as their removal) by direct smear under the compound microscope (100x). The material from the first cecum examined which contained oocysts, was placed in tap water and treated as described under preparation of cultures. These oocysts

constituted the so-called "pure line strain" which was used throughout this experiment. Both species were treated in a like manner.

6. Preparation of Culture

On the eighth or ninth day post infection, enough remaining birds were sacrificed (in their respective rooms) to obtain a quantity of oocysts necessary for the next "run." Usually for E. tenella this required only five to ten birds for more than an adequate supply. For E. necatrix fifteen to twenty birds were needed to collect slightly over the amount of oocysts needed.

In collecting E. tenella oocysts, the ceca were removed from the sacrificed birds and cut lengthwise. The contents were removed and placed into a screw cap jar (100 ml. capacity) which was one-half filled with tap water. The cecal epithelium was not scraped and it was hoped that the number of nonfertile oocysts were thus kept at a minimum.

With E. necatrix infected and sacrificed birds, the ceca were never cut open for oocyst collecting purposes. Since no cores ever formed and the ceca walls were rarely enlarged, the material inside the ceca was forced through a cut in the distal end of the blind sack. By holding the ceca against the rim of the jar with the fingers of one hand and grasping the distal or constricted end of the ceca in the other, the ceca would be pulled through this constriction

thus forcing the cecal fluid into the water inside the screw cap jar. The oocyst collection of both species was never made on the same day.

This material was brought back to the laboratory and placed in a metal "Waring Blendor" jar and subjected to the blender's agitation for one to three minutes. The ceca cores from birds infected with E. tenella required more time than the material collected from the ceca of E. necatrix infected birds.

The material from the blender jar was poured into two 100 ml. round bottom centrifuge tubes and centrifuged at approximately 1000 rpm for one to one and one-half minutes. The supernatant was poured off and the tubes refilled with tap water. This procedure was repeated until the supernatant was clear (usually four or five washings). The supernatant was then removed and a two percent potassium dichromate solution (15-25 ml.) added to one tube. This tube was thoroughly shaken and the suspension poured into the other tube, which was shaken in a similar manner. The combined material from these tubes was poured onto a series of sieves (sieve sizes National Bureau of Standards Series of 100, 200, and 325). The material, whose diameter exceeded approximately 44 μ was retained and the smaller material, including the oocysts, passed through and collected in a 250 ml. Erlenmeyer flask beneath the funnel holding the screens. Fifty to one hundred ml. of two percent potassium dichromate solution was poured

onto the screens to wash through as many oocysts as possible.

The material collected in the flask was poured into sterile petri dishes (each filled one-quarter to one-third from the bottom) and the oocysts were allowed to sporulate (three to four days) at room temperature. Once each day during sporulation, the material in each petri dish was agitated with a clean applicator stick.

All of the equipment used in this procedure, except the culture and petri dishes, was autoclaved for at least ten minutes at 248° F. and sixteen lbs. of steam pressure. All laboratory equipment used in subsequent procedures with these pathogens was likewise autoclaved before being used again.

On the third or fourth day after collection, the culture was pipetted from the petri dishes into centrifuge tubes and centrifuged (as above) until all potassium dichromate solution was removed. Tap water was added to one tube which was then shaken and poured into the other tube. This tube was shaken and the culture poured into a 100 ml. prescription bottle and the culture diluted with tap water to the 40-60 ml. level. This bottle, containing the culture ready to use, was placed into the walk-in refrigerator (2° C) until needed for the next "run," which in most cases was three to five days after being placed in refrigerator storage.

7. Oocyst Counts

After removal from the walk-in refrigerator, the culture was taken to the laboratory. The bottle was shaken vigorously for approximately one to two minutes in an up and down, as well as a rotating manner, in order to get the oocysts evenly distributed throughout the medium. Immediately after this a W.B.C. (white blood cell) pipette (with rubber tubing and mouth piece in position) was inserted into the middle of the culture suspension. Material was withdrawn up to the bottom of the W.B.C. pipette bulb and transferred immediately under a hemocytometer cover slip (both sides) which was in position on the hemocytometer counting chamber (Spencer Bright Line). No dilution factors were involved, the W.B. C. pipette used merely as a means of transfer.

Using the areas of the counting chamber, which are normally used for W.B.C. counts, the number of sporulated oocysts were counted. All eight of these areas were utilized in each count, thus making a total area of eight square millimeters (mm^2). To find the number of sporulated oocysts per one mm^2, the total number counted was divided by eight (e.g., if 80 sporulated oocysts were counted in eight mm^2, then there were 80/8 or 10 sporulated oocysts per mm^2). To get volume (cubic millimeter or mm^3), the number of oocysts per mm^2 was multiplied by ten since the distance between the cover slip and counting chamber is 0.1 mm. To get the number of sporulated oocysts in one milliliter* (ml.), the number of oocysts

*One (ml.)equals approximately one cubic centimeter (cc.).

per mm^3 was multiplied by 1000, since 1 mm^3 equals approximately 0.001 ml. Thus in the examples used above we have:

$$\frac{80 \text{ oocysts per } 8 \text{ mm}^2}{8} \times 10 \text{ to cube} \times 1000 \text{ conversion factor} = 100{,}000 \text{ oocysts /ml.}$$

or

$$\frac{\text{number of oocysts per } 8 \text{ mm}^2}{\text{mm}^2 \text{ utilized}} \times 10{,}000 = \text{number of oocysts/ml.}$$

On the basis of the above count the necessary dilution was calculated in order to get the desired number of oocysts per ml. (e.g, if there were 100,000 sporulated oocysts per ml. in the culture and a suspension of 20,000 per ml. was desired, by dividing 100,000 by 20,000 a factor 5 is obtained. This number 5 means that there are 5 groups of 20,000 oocyst in 1 ml. containing 100,000. By diluting 1 ml. containing 100,000 sporulated oocysts with 4 ml. of tap water we obtain 5 ml. each of which contains 20,000 sporulated oocysts. If 30 ml. of suspension containing 20,000 sporulated oocysts per ml. is desired, the following calculation is made:

$30 \div 5 = 6$

$1 : 4 \times 6 = 6 : 24 = 30$

In these experiments three different dosages were used in each series of "runs," so that three checks were made on the original culture count. If any one of these varied more than 5,000 in either direction (e.g., 20,000 $\pm$ 5,000) rechecks were made on the dilutions and original count until all

dilutions, when calculated from the original, fell within the desired range when counted as above.

In all cases the oocyst suspension was prepared in screw cap vials of a size which best suited the total number of ml. desired for each run (e.g., if 10 ml. were needed at least 11 ml. were prepared in a 25 ml. capacity vial, if 30 ml. were desired, a 45 ml. capacity vial was used and at least 31 ml. were prepared.

8. Administration of Oocysts

The vials containing the proper number of occysts per ml. were taken to the animal housing area. These vials were taken to their respective rooms. Each bird was given 1.0 ml. of the desired inoculum and placed in the brooder as follows: lowest number of oocysts per bird group on the top level, and proceeding downward with the other groups so that the group of birds receiving the greatest number of oocysts per bird was on the bottom level.

The inoculum was administered with an Exax serological pipette (1 ml. capacity). This pipette was inserted into the desired inoculum, filled and exhausted 8 times, filled to above the 0.0 mark and the culture was held at that position in the pipette by holding the index finger of the right hand over the pipette bore. The material was carefully allowed to run down to the 0.0 mark. The pipette was removed and placed into the crop by holding the bird in the left hand, grasping the beak with the thumb and index finger, the mouth

was forced open, the pipette inserted into the esophagus and at the same time the neck was stretched out by placing the butt of the left hand on the dorsal thorax area and extending the thumb and index finger holding the beak straight up and the pipette pushed into the crop. When properly inserted, the index finger holding the inoculum in the pipette was released and the inoculum drained into the crop. As the pipette was being withdrawn, the remaining culture was blown out with air from the mouth.

In order to avoid the possibility of carrying over a large number of oocysts from a vial containing a larger number of oocysts to one with a lower number, the bird group receiving the lowest number of oocysts per inoculum were the first to be infected, followed by the group which received the next highest number, and so on until all were infected.

In order to help keep accidental contamination of either of the two species with each other occurring, their inoculation occurred on separate days, e.g., birds were infected with E. tenella on Monday and with E. necatrix on Tuesday. Mixed infections were carried out using the same vials (different pipettes) as those used in pure infections and usually this group of chicks received its inoculation on the same day as the second "pure" group. In the example above, this would occur on Tuesday, A separate vial of "pure" inoculum was prepared at the same time as the other vials for studies with Aureomycin. The birds used in these

experiments were inoculated after the birds which were used to maintain pure oocysts. Vials and pipettes were autoclaved after being used.

9. Dosage of Oocysts

E. tenella: The range of inocula given varied from 20,000 sporulated oocysts to 140,000 per bird. However, of the eighteen runs made, ten were as follows: Group I--20,000, Group II--40,000, Group III--80,000 sporulated oocysts per bird.

E. necatrix: Range of inoculum given varied from 20,000 to 360,000 sporulated oocysts per bird. As with E. tenella, of 18 runs made, 10 were made at the levels of 20, 40, and 80 thousand per bird.

Mixed infection: In preliminary runs the number of oocysts given per bird was as follows: E. tenella, 20,000 to 140,000; E. necatrix, 50,000 to 280,000. Five runs were made where the three groups of ten birds received 40,000 E. necatrix oocysts and Group I received 20,000; Group II received 40,000; and Group III received 80,000 E. tenella oocysts. Five runs were made where the total numbers involved were the same as above except the oocyst number of E. necatrix was varied and that of E. tenella was constant at 40,000.

10. Feed

The feed used throughout the entire experiment was non-antibiotic, non-medicated chick starter mash. It was

supplied by the Valley City Milling Company, Portland, Michigan. Its composition was as follows:

Lbs. per mix	Ingredients	Total lbs.
1820	Ground yellow corn	455
400	Pulverized oats	100
2	Delsterol	0.5
280	4 percent soybean oil meal	220
20	Red fish meal	5
200	Meat scraps	50
400	Middlings	100
100	17 percent alfalfa meal	25
60	Poultry mineral A	15
80	Ground fermentation solubles	20
40	Condensed fish solubles	10
5	Vitamin A Premix	1.25
10	Salt	2.5
0.5	Manganese Sulphate	2 oz.
	B12 supplement	2/3 lbs.
	Choline chloride	1/2 lb.
		1005 13/24

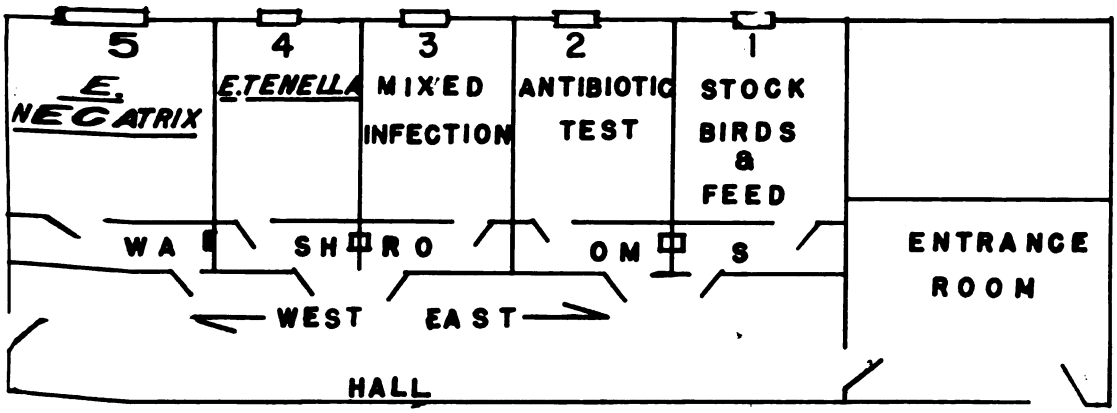
For the antibiotic study, this same feed was supplemented with Aureomycin in the following manner. Three separate weights (2.5, 5 and 10 grams) of Aureomycin were weighed on an analytical balance and placed in small separate screw cap vials. Three eight-gallon cans were each filled with twenty five pounds of the above feed. The feed from one can was placed in a small electric cement mixer. A pre-mix was then prepared using a small amount of feed from the cement mixer and the antibiotic from one vial. The mixer was turned on and the pre-mix was added slowly to the feed and allowed to mix for fifteen minutes.

The feed from each can was treated as above. The lowest level of Aureomycin mixed first and the highest level

last. The mixer was then washed with hot water and allowed to dry before re-using.

11. Housing and Utensils

One-half of the west wing (five isolated rooms) of the M.S.U. animal disease barn No. 5 was used throughout these experiments. A diagram of this area and how each room was utilized is as follows:



Each room contained the following:

1. Brooder. Rooms 1 and 2 each contained one, five-level brooder. Each level had its own heating unit and thermostat. Rooms 3, 4 and 5 each contained one three-level brooder. Each level had its own heating element, but one thermostat controlled all three levels.
2. Feed can. Eight gallon capacity.
3. Fecal can. Eight gallon capacity.
4. Combination scrub brush and squeegee.
5. Paint scraper.
6. Thermometer (-10° to 120° C.).

7. Knee boots.

8. Data book and pencil.

9. Coveralls.

The fecal can and coveralls were the only things which were ever removed from these rooms.

B. The Effect of Aureomycin on the Sporozoite and Merozoite Stages of E. tenella

The same type of chicks and feed as were used in the pure and mixed infection studies were utilized in this study. Chicks used in Aureomycin versus mortality studies (Table V) were handled and infected as previously mentioned under pure and mixed infections. The exception being that three groups of chicks were placed on feed containing Aureomycin at the levels of 5, 10 and 20 grams of Aureomycin per 50 pounds of feed respectively. These were mixed as follows: a premix was made, which was then added slowly to the feed in a small electric cement mixer. The inoculum, in all cases, was 40 thousand sporulated oocysts per bird for E. tenella infection and 80 thousand sporulated oocysts per bird with E. necatrix infection. The control birds were the same as those used for mixed-infection studies (denoted as passage number).

For antibiotic studies, chicks (varying in age from one to six weeks) were brought into the laboratory area via the original carton in which they were sent from the hatchery. In the laboratory they were maintained in this carton without

feed or water for two to three hours. Fifteen older birds (four to six weeks of age) were then inoculated as follows: five chicks received approximately one million sporulated oocysts. The remaining ten received approximately 20 thousand sporulated oocysts. This latter group of birds was placed in a chicken cage, fed, watered and kept separated from the former since the five receiving one million oocysts each were sacrificed after one hour for sporozoite collecting purposes. This collection was carried out by removing the small intestine (from the gizzard to the ceca) cutting it open throughout its entire length and immersing the intestine into a buffered (Sorensen's phosphate buffer-pH 7.0) saline solution. When all five birds had thus been treated, the intestines were thoroughly agitated in and then removed from the saline solution. The saline was then poured through a series of sieves (sieve sizes National Bureau of Standard Series of 100, 200 and 325) and collected beneath a large funnel in an Erlenmeyer flask (250 ml.). This material was poured into two 100 ml. round bottom centrifuge tubes and centrifuged at approximately 1000 rpm for one to two minutes and the supernatant was poured off. Using a 5 ml. pipette, the sediment was withdrawn and distributed in equal amounts in three sterile petri dishes. To the first petri dish, 10 ml of buffered saline were added. to the second, 10 ml. of a .022 percent Aureomycin in buffered saline solution and to the third a .044 percent solution of Aureomycin buffered saline. Material was withdrawn from each

of the three petri dishes at time intervals of 5, 15, 30, 60 and 120 minutes with separate 2 ml. pipettes. Two ml. of the material was removed via individual pipettes and transferred to 15 ml. conical centrifuge tubes (one for each of the three testing materials), and centrifuged for one minute at 900 rpm. The supernatant was poured off and to each tube were added 2 ml. of buffered saline and the sediment and saline thoroughly mixed. Using a clean 2 ml. pipette for each of the three tubes, 2 ml. of the material was withdrawn from each tube. Holding a chick upside down and with the tail up and away from the body, the tip of the pipette was inserted into the cloaca about one-quarter of an inch. One ml. of the material was then blown into the cloaca. As the pipette was being withdrawn, the cloaca was pinched shut with the thumb and index finger and held shut for about one or one and one-half minutes to enhance the retention of this fluid. Fluids thus injected go almost immediately to the ceca (Dukes, 1955). Another bird was similarly given the remaining ml. and both were properly banded for future observations. This injection method was used throughout each time interval and for each test material. This method is similar to tnat described by Levine (1940) for cloacal merozoite infection.

Sporozoite and merozoite collections, as well as treated and untreated collection material and the inoculation of the birds, were carried out in a walk-in incubator (37° C).

Chicks were removed from the incubator immediately after inoculation and maintained in the same carton in which they were brought into the laboratory. No food or water was supplied to the birds receiving sporozoites until the following day in order to avoid contamination. Merozoite-infected birds were immediately removed to cages after inocula and given feed and water.

Other control chicks were treated as follows: 1 ml. of buffered saline was given by cloaca to two birds for each time interval, and two chicks received no inocula but were maintained with the other chicks at all times. Two chicks were given 1 ml of sporulated oocyst by cloaca while two others were given 1 ml. of a mixture of oocysts and spores. The latter mixture was obtained by subjecting a sporulated oocyst suspension to the action of an all-glass tissue homogenizer for a very short time. Attempts were made to avoid releasing sporozoites which had occurred due to rapid and prolonged action. Although no sporozoites were observed under a microscope, the possibility does exist that a few were released.

Merozoites stages (Generation I and II) were collected from the ceca on the third and fifth days post infection, respectively, and treated in a similar manner as the sporozoites previously described.

On the seventh day post infection, since this type of infection does not alter the duration of the life cycle

(Levine, 1940), all birds were sacrificed and examined for oocysts as described under single oocyst infection collection. In the case of negative results (no oocysts) the contents of the ceca of these birds were checked by the sugar flotation method described under pure and mixed infections.

In the second experiment, where one level of Aureomycin was substituted with a higher level, the centrifugation (as before) of Generation I merozoites was increased to 2000 rpm. Since no infections occurred with this stage in the first experiment, the possibility existed that these merozoites were not in the sediment when subjected to 1000 and 900 rpm due to their small size (2-4 μ x 1-1.5 μ) as compared with the size (16 μ x 2 μ) (Tyzzer, 1929) of the Generation II merozoites.

As with pure and mixed infection studies, utmost care was exercised to prevent accidental infection with any oocysts. Since E. tenella merozoites (Generation II) failed to cause infection when introduced via the crop (Levine, 1940), passed feces presented no problem with this stage of infection. The other two stages have not previously been reported upon when treated in a similar manner. Since with sporozoite infection the possibility exists for both oocysts and spores to be present with the sporozoites as collected, no oral inocula were made. However, oral inocula with Generation I merozoites were attempted.

Birds were supplied feed and water ad libitum after inoculation, exceptions noted previously.

RESULTS

A. Single Oocyst Isolation, Pure and Mixed Infections

Of a total of ten two-week old chicks, each of which received one E. tenella sporulated oocyst, only one, the ninth bird examined, had become infected (oocysts were demonstrated by direct smear). Only one of the two ceca contained oocysts. A similar group of ten chicks, each of which received one E. necatrix sporulated oocyst, yielded, on the fourth bird examined, oocysts in both ceca of this same bird.

Preliminary investigations with sporulated oocysts of E. tenella at an inoculum level varying from 20 to 140 thousand per bird, indicated that 40 thousand (± 5 thousand) would cause approximately 50 percent mortality. Infections with sporulated oocysts of E. necatrix in passages 3 through 7 gave results which indicated a very erratic type of infection. The oocysts collected in passage number 7 had to be used in three different "runs" due to insufficient oocyst collection. However, average results of mortality (0-100 percent) indicated that 50 to 70 thousand sporulated oocysts would produce approximately 43 percent mortality. Thus, on the basis of these results, 80 thousand sporulated oocysts should produce approximately the same mortality as E. tenella at the 40 thousand level. For further "runs" with pure and

mixed infections, the inocula levels of 20, 40 and 80 thousand sporulated oocysts per bird were used.

Since mixed infection studies require controls which could also be used to determine if the 40 and 80 thousand inocula of E. tenella and E. necatrix would produce an LD_{50}, both experiments were run as one unit.

Table I shows the results of five "runs" where, in the mixed infections studies, the inocula of E. tenella oocysts were varied, while the inocula of E. necatrix oocysts were constant. Table II shows the results of five "runs" where (with respect to dosages in mixed infections) the converse is true.

The average of these ten "runs" with respect to each other (I-V compared with VI-X) is given in Graph I.

A comparison of the relationship between the average percent mortality caused by mixed infection and the average percent mortality caused by the total of both species of pure infection is given in Table III.

In an attempt to determine if mixed infection caused any change in mortality with respect to the number of days after infection when compared with that of either of the two pure strains, mortality dates were recorded and are listed in Table IV.

TABLE I

PERCENT MORTALITY OF CHICKENS* WITH PURE INFECTIONS OF E. TENELLA AND E. NECATRIX AND WITH MIXED INFECTIONS WHERE THE NUMBER OF OOCYSTS PER BIRD WAS VARIED FOR E. TENELLA AND CONSTANT FOR E. NECATRIX**

	Runs	I			II			III			IV			V		
Eimeria tenella	Passage Number	9	9	9	10	10	10	11	11	11	12	12	12	13	13	13
	Age of birds in days	14	14	14	17	17	17	15	15	15	14	14	14	14	14	14
	Thousands of oocysts per bird	20	40	80	20	40	80	20	40	80	20	40	80	20	40	80
	Percent mortality	30	40	10	30	30	60	10	30	10	10	40	40	0	40	80
Eimeria necatrix	Passage Number	7	7	7	8	8	8	9	9	9	10	10	10	11	11	11
	Age of birds in days	15	15	15	18	18	18	16	16	16	15	15	15	15	15	15
	Thousands of oocysts per bird	20	40	80	20	40	80	20	40	80	20	40	80	20	40	80
	Percent mortality	0	0	10	0	40	100	0	10	50	0	10	10	0	0	10
Mixed Infection	Passage Number	As above														
	Age of birds in days	15	15	15	18	18	18	16	16	16	15	15	15	15	15	15
	Thousands of oocysts per bird, E. tenella	20	40	80	20	40	80	20	40	80	20	40	80	20	40	80
	Thousands of oocysts per bird, E. necatrix	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
	Percent mortality	40	70	30	40	80	100	90	100	90	40	40	50	30	80	90

*Ten birds in each group.
**Ten control birds, for each run, given 1 cc. of tap water, had no deaths nor were oocysts passed.

TABLE II

PERCENT MORTALITY OF CHICKENS* WITH PURE INFECTIONS OF *E. TENELLA* AND *E. NECATRIX* AND WITH MIXED INFECTIONS WHERE THE NUMBER OF OOCYSTS PER BIRD WAS VARIED FOR *E. NECATRIX* AND CONSTANT FOR *E. TENELLA***

	Runs	VI			VII			VIII			IX			X		
Eimeria tenella	Passage Number	14	14	14	15	15	15	16	16	16	17	17	17	18	18	18
	Age of birds in days	13	13	13	14	14	14	15	15	15	15	15	15	15	15	15
	Thousands of oocysts per bird	20	40	80	20	40	80	20	40	80	20	40	80	20	40	80
	Percent mortality	20	40	60	30	70	60	10	30	40	20	60	30	10	10	20
Eimeria necatrix	Passage Number	12	12	12	13	13	13	14	14	14	15	15	15	16	16	16
	Age of birds in days	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
	Thousands of oocysts per bird	20	40	80	20	40	80	20	40	80	20	40	80	20	40	80
	Percent mortality	0	40	60	0	30	100	0	0	10	0	20	30	10	0	10
Mixed Infections	Passage Number	As above														
	Age of birds in days	14	14	14	15	15	15	15	15	15	15	15	15	15	15	15
	Thousands of oocysts per bird, *E. tenella*	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
	Thousands of oocysts per bird, *E. necatrix*	20	40	80	20	40	80	20	40	80	20	40	80	20	40	80
	Percent mortality	80	60	90	50	80	90	10	30	80	50	50	60	40	50	30

*Ten birds in each group.

**Ten control birds, for each run, given 1 cc. of tap water, had no mortality nor were oocysts passed.

GRAPH I

COMPARISON OF AVERAGE MORTALITY RATES WITH PURE AND MIXED INFECTIONS OF RUNS I - V WITH RUNS VI - X

⊙—⊙ Mixed infection (*E. tenella* inocula varied at 20, 40 and 80 thousand)
(*E. necatrix* inocula constant at 40,000)
X—X Control (*E. tenella* pure infection)
□—□ Control (*E. necatrix* pure infection)

Dotted line denotes the second experiment where the *E. necatrix* oocysts inocula was varied -- *E. tenella* constant at 40,000 oocysts per bird per group.

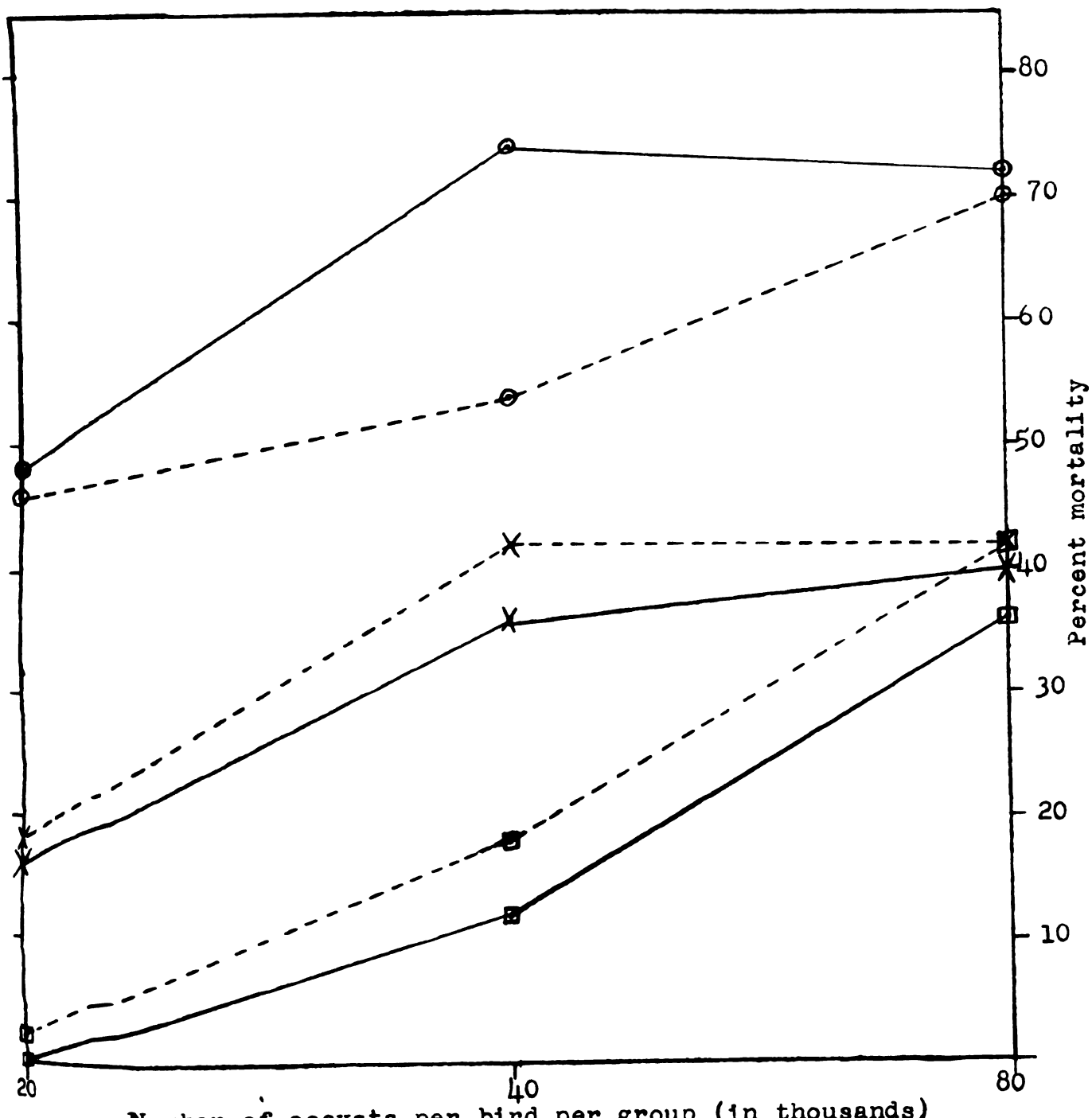


TABLE III

COMPARISON OF THE AVERAGE MORTALITY CAUSED BY PURE AND MIXED INFECTION

Inocula Level	Average Mortality in Percent			
	E. tenella	*E. necatrix*	Total	Mixed Infection
	Runs I - V			
20,000	17		29	48
40,000	39	12*	51	74
80,000	41		53	72
	Runs VI - X			
20,000		2	44	46
40,000	42**	18	60	54
80,000		42	84	70

*In runs I-V, 40,000 oocysts of *E. necatrix* were given to chicks along with the three levels of *E. tenella* oocysts. In the totals the average percent mortality caused by this inocula level is included with those caused by the three levels of *E. tenella*.

**The converse is true for runs VI-X.

TABLE IV

RELATIONSHIP BETWEEN THE TOTAL NUMBER OF DEATHS AND THE DAYS AFTER INFECTION ON WHICH THESE OCCURRED*

Days After Infection	3	4	5	6	7	8	9	Total Deaths
Deaths due to *E. tenella*	0	0	64	92	18	0	0	174
Percent	-	-	36.5	53.5	10	-	-	
Deaths due to *E. necatrix*	0	2	62	54	14	0	0	132
Percent	-	1.5	47	41	10.5	-	-	
Deaths due to mixed infection	0	0	41	154	29	7	0	231
Percent	-	-	18	67	12	3	-	

*Preliminary data included with that in Tables I and II.

B. The Effect of Aureomycin on the Sporozoites and Merozoite Stages of *E. tenella*

The experiments to determine what effects various levels of Aureomycin in the feed might have on chick mortality are tabulated in Table V. The controls in these experiments correspond to those used in mixed infection studies and are so designated by the passage numbers which are the same as the number of serial passages after single oocyst isolation.

The results of *in vitro* experiments of Aureomycin, at the same levels as per the mortality studies, on the sporozoite and merozoite stages of *E. tenella* are recorded in Table VI.

TABLE V

THE EFFECT OF VARIOUS LEVELS OF AUREOMYCIN IN THE FEED ON THE MORTALITY OF CHICKS INFECTED WITH E. TENELLA AND E. NECATRIX

Run Number	Passage Number	Age of Chicks (days)		Grams of Aureomycin per 50 pounds of Feed			Controls
				5**	10	20	
1. Pure E. tenella infections with medicated feed given to chicks (10 per group) the day before infection. (40,000 sporulated oocysts per bird)							
XI	11	18	Percent Mortality	50	0	0	30
XII	12	18		40	0	0	40
XIII*	13	16		40	10	0	40
2. Pure E. necatrix infections with medicated feed given to chicks (10 per group) the day before infection. (80,000 sporulated oocysts per bird)							
XIV	13	15	Percent Mortality	30	0	0	100
XV	14	14		10	0	0	10
XVI	15	16		10	0	0	30

*Antibiotic feed given to chicks the same day as infection.
**Five grams of Aureomycin per 50 pounds of feed = .022 percent.

TABLE VI

IN VITRO STUDIES OF THE EFFECT OF AUREOMYCIN ON THE SPOROZOITE AND MEROZOITE (GENERATIONS I AND II) STAGES OF *EIMERIA TENELLA*

	Controls*									Aureomycin .022%					Aureomycin .044%									Aureomycin .088%			
Time in hours	1/12	1/4	1/2	1	2	2	4	8	16	1/12	1/4	1/2	1	2	1/12	1/4	1/2	1	2	2	4	8	16	2	4	8	16
SPOROZOITE																											
Number of birds inoculated	1	1	1	1	1	2	2	2	2	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
Number of birds with oocysts	1	1	1	1	1	2	2	2	0	1	1	1	1	1	1	1	1	1	1	2	2	0	0	0	0	0	0
MEROZOITE I																											
Number of birds inoculated	1	2	2	2	2	2	2	2	2	1	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2
Number of birds with oocysts	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MEROZOITE II																											
Number of birds inoculated	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Number of birds with oocysts	2	1	1	1	1	2	0	0	0	2	1	2	0	1	1	2	1	1	1	2	0	0	0	0	0	0	0

*Other controls:

1. Two chicks received sporulated oocysts cloacally. No oocysts seen at the end of 7 days.
2. One chick received sporulated oocysts and spores via the cloaca. No oocysts were seen at the end of 7 days, but Generation II merozoites were seen in great numbers.
3. Two chicks received 1 cc. of buffered saline cloacally. No oocysts seen at end of 7 days.
4. Twelve chicks (two for each group of stages) were uninoculated. No oocysts seen after 7 days.
5. Two chicks received inocula of Generation I and II merozoites via the crop. No oocysts were seen 7 days after inoculation.

DISCUSSION

A. Pure and Mixed Infections

1. Pure Infections

The results show that the LD_{50} for E. tenella was never realized with 40 thousand sporulated oocysts per bird. However, at this level of inoculum, the mortality rate appears to reach a maximum and at the higher level of 80 thousand oocysts, no significant difference* in mortality was observed. At the 20 thousand level there is a significant difference in mortality as compared with the 40 and 80 thousand level. When comparing the results of the two groups of "runs" ("runs" I through V with VI through X), with E. tenella, there was no significant difference in the mortality at the three levels of infection (Graph I). Some explanation for the range of 10 to 80 percent mortality may be explained in difference in inherent resistance of the host (Herrick, 1934). Virulence of oocysts may also offer some explanation, however rapid passages of oocysts were undertaken to keep variations in virulence at a minimum.

The results of these experiments do not substantiate the report by Gardiner (1955) that two-week old birds are resistant to high mortality rates with E. tenella infections. Although Gardiner did not mention the breed of chickens which

*Statistical analyses were made and significance was based on the 5 per cent level.

he used, it is possible that this difference can be accounted for by breed differences as indicated by Rosenberg (1941).

E. necatrix infections show a progressive increase in mortality with inocula levels of 20, 40 and 80 thousand sporulated oocysts. It would appear from these results (Graph I) that the maximum mortality has not been realized. It is interesting to note that E. necatrix infection at the 80 thousand level was approximately the same as that found with E. tenella at both the 40 and 80 thousand level. Furthermore, there is no significant difference between the mortality of E. tenella 20 thousand oocysts level and the 40 thousand E. necatrix level. At the 20 thousand level of both species E. tenella causes eight times the number of deaths as does E. necatrix. Tables I and II show that the mortality at the 40 and 80 thousand level with E. necatrix are very erratic, ranging from 0 to 40 percent at the 40 thousand level and from 0 to 100 percent at the 80 thousand level. These extremes occurred more than once in the ten "runs." While not enough "runs" were completed with this species to produce conclusive results, there is some indication that high mortality (50-100 percent) occurred in cycles. For example, the 4th (preliminary "run"), 7th, 8th, 12th and 13th passages produced mortalities of 50 percent or greater at the 80 thousand level. Thus it appears that two successive passages produce high mortalities, while the next three or four produce less than 50 percent.

As with E. tenella, the two groups of "runs" with E. necatrix show no significant difference from each other at the three levels of inocula.

The evidence of this work agrees with that of Brackett and Bliznick (1952) that young birds are susceptible to E. necatrix infection. Furthermore, at the 80 thousand level this pathogen killed all the birds on two different occasions while E. tenella never killed more than 80 percent and this occurred only on one occasion. Thus it appears that of the two species studied E. necatrix is the more pathogenic at high levels, being able occasionally to kill 100 percent of the birds. However, below the 80 thousand level E. tenella averaged a higher mortality than E. necatrix at the same level. Also E. tenella on occasion caused a 60 and 70 percent mortality at the 40 thousand level while E. necatrix at the same level never exceeded 40 percent.

2. Mixed Infections

Results from mixed infection studies show that the average percent mortality caused by both species in combination, at the levels studied were approximately the same as, or greater than, the average percent mortality caused by both species of pure infection at the same level (Table III). For example, in mixed infection with the E. tenella inocula at the 40 thousand level and E. necatrix inocula at the 20 thousand level the average mortality was 46 percent. The average

mortality at the 40 thousand level for pure infection of E. tenella was 42 percent and at the 20 thousand level for the pure infection of E. necatrix, 2 percent, making a total of 44 percent mortality.

Evidence is also presented which shows that the mortality was approximately the same for mixed infections regardless of which species was varied or kept constant. However, in the mixed infections at the level of 40 thousand oocysts of both species, a significant difference of the average mortality was noted between the two experiments (Graph I). One explanation that may be significant is that during run III (Table I) the temperature in the room housing the chicks (Mixed infection) rose to 35° C on the sixth day after infection. This, of course, may have increased the mortality at all three levels, which could account for the overall higher average at all levels of mixed infection in runs I through V.

Since in mixed infection of the total number of oocysts per inoculum at each level, was 60, 80 and 120 thousand respectively, a comparison of mixed infections at the 40 thousand E. tenella and E. necatrix level can be made with the 80 thousand level pure infection of both species. However, the runs (I-V) in which E. tenella oocyst level was varied and the E. necatrix level constant, produced a higher average percent mortality (74 percent) at the 80 thousand level (40 thousand of each species) than did the mortality (54 percent) obtained in the converse runs (VI-X). These dissimilar percent

mortalities cannot be combined and averaged because they are significantly different from each other. However, in the runs where the E. tenella level was varied (Table I) there is a significant difference at the 80 thousand level (40 thousand of each species) when compared with the 80 thousand level of pure infection. Why in one group of runs a mixed infection of equal total numbers of oocysts per inocula is significantly different from an equal number of pure inocula and not in the other group of runs of similar inocula is not known. Perhaps the key to this solution rests with the previously mentioned fact that the room temperature rose very high in run number III, which may have affected mortality.

Utilizing Graph I and estimating the number of deaths caused by an inocula of 60 thousand oocysts for E. necatrix it is found to be approximately 27 percent. In a mixed infection at the 40 thousand E. necatrix level and 20 thousand E. tenella level the average mortality is 47 percent. These mortalities are significantly different from each other and give further evidence that at certain levels mixed infections cause a higher percent mortality than do pure infections of equal numbers of oocysts. More conclusive evidence may have been obtained if the 20 thousand level for E. tenella had been used in place of the 40 thousand level used in mixed infections during runs VI-X, since the 40 thousand level of E. tenella appears to cause maximum mortality in two-week old chicks.

The results of the relationship between the total number of deaths and the days after infection on which these occurred show that neither the pure nor mixed infections caused deaths on the third or ninth day post infection. Evidence is also presented which shows, that while the majority of deaths in pure and mixed infection occurred on the 5th, 6th and 7th day after infection, deaths occurred on the 3rd day with E. necatrix and on the 8th day post infection with mixed infections. The greatest number of deaths occurred as follows: E. tenella on the 6th day, E. necatrix on the 5th and 6th days, and mixed infections on the 6th day post infection.

B. The Effect of Aureomycin on the Sporozoite and Merozoite Stages of E. tenella

The results in Table V show that Aureomycin at the .044 and .088 percent level prevent mortality with E. tenella and E. necatrix infections when administered the day before infection. When medicated feed was given the same day as inocula, mortality was not prevented at the .044 percent level but was at the .088 percent level (E. tenella). Seegar et al. (1950), using a 0.1 percent mixture of Aureomycin and feed (2 pounds Aureomycin per ton of feed) found that mortality caused by E. tenella was prevented when birds were fed continuously. These same authors found that a 0.2 percent level of antibiotic fed 48-120 hours after inoculation, did not prevent mortality. Two hundred grams of Aureomycin per

ton of feed (.022 percent) administered the same day as infection, reduced chicken mortality (Gardiner, 1957).

In E. necatrix infection there is some evidence that 5 grams of Aureomycin per 50 pounds of feed (.011 percent) reduces mortality. At least two explanations are possible: (1) Less time is required for markers to appear in the feces of birds treated with Aureomycin than those not treated, which may be due to stimulation of intestinal contractions (Jukes et al., 1956). Thus some sporozoites may have passed through the small intestine area before they could become established. (2) When Auroemycin is administered orally, it is present in relatively high concentrations in the upper and lower small intestine and low in the ceca (Yacowitz and Bird, 1953). Therefore, E. necatrix stages were subjected to a higher concentration of Aureomycin than those of E. tenella which gave no indication of reduced mortality at this level.

The results of the in vitro studies with Aureomycin show that sporozoites, when subjected to a .044 percent solution, are inactivated somewhere between 4 and 8 hours. With a .088 percent solution of Aureomycin, they are inactivated sometime before 2 hours. If chick mortality was, in part, prevented by Aureomycin action against sporozoites, as indicated by run XIII, we can assume that some sporozoites were subjected to Aureomycin for at least 2-4 hours (Table VI). This indicates that sporozoites may not all enter cells at

the same time and is in agreement with a similar suggestion by Tyzzer (1929).

Sporozoites failed to initiate infection after 16 hours in buffered saline at 37° C.

Generation I merozoites did not cause any infection, under the conditions of this experiment, as was indicated by lack of oocyst development. Why? Three possible explanations are: (1) This stage may not occur in the lumen of the ceca on the third day after infection in great numbers. (2) These merozoites may not occur in the lumen of the ceca in great numbers at any time and thus were not collected. (3) This stage may be non-resistant to the conditions under which the experiments were conducted.

Generation II merozoites failed to initiate infection after 2 hours in buffered saline at 37° C. No activity against this stage by a .044 percent solution of Aureomycin was noted up to this time. Nevertheless, .088 percent solution of the antibiotic inactivated this stage on or before 2 hours. As noted in Table VI, infections with this stage were not as consistent as were those infections initiated by sporozoites. This is understandable since sporozoites go through two asexual stages which results in great numbers of oocysts, while merozoites of Generation II, except for microgametes, do not result in any increase in numbers.

Under the conditions of this experiment, the sporozoite stage was four times as resistant as the Generation II

merozoites when placed in buffered saline at 37° C. No data are available concerning the resistance of the Generation I merozoites, but the possibility exists that they are the least resistant of all three stages.

It is noted that the cecal contents of the control (1 chick) which received sporulated oocysts and spores via the cloaca, contained no oocysts, but had numerous Generation II merozoites. Such results are possible because: (1) Sporozoites may have been present in the suspension as previously mentioned, although one would anticipate oocysts at the end of seven days had this occurred. (2) This bird was maintained in an isolated rat cage, the bottom of which was covered by newspaper, and it is highly possible that this chick had eaten passed feces which contained oocysts and spores (those inoculated).

SUMMARY

A. Pure and Mixed Infections

Mortality rates were investigated with pure and mixed infections of Eimeria tenella and Eimeria necatrix. This is believed to be the first report of studies with mixed infections with known amounts of sporulated oocyst inocula.

B. The Effect of Aureomycin on the Sporozoite and Merozoite Stages of E. tenella

Investigations were carried out to determine if Aureomycin incorporated in the feed at the levels of 5, 10 and 20 grams per 50 pounds of feed (.022, .044 and .088 percent, respectively) would affect mortality of birds infected with E. tenella and E. necatrix.

Sporozoites and merozoites (Generations I and II) were collected from chickens artificially infected with E. tenella. In vitro tests were made to determine if Aureomycin, at the same levels as mortality studies above, affected these stages and if so, the time required for this effect to take place.

This is believed to be the first reported in vitro test employing sporozoite and merozoite stages.

CONCLUSIONS

A. Pure and Mixed Infections

1. Eimeria tenella infection caused a high mortality rate with two-week old Vantress Cross (White Rocks) chicks.

2. E. tenella infection caused the maximum average percent of mortality (39 and 41) at the 40 and 80 thousand oocyst levels studied.

3. Eimeria necatrix infection caused a high mortality with similar birds.

4. E. necatrix infection caused the highest percent mortality at the 80 thousand sporulated oocyst inocula level per bird with respect to the 20, 40 and 80 thousand oocyst levels studied.

5. There is some indication that the maximum percent mortality was never realized with the oocyst inocula levels used in the E. necatrix studies.

6. Mortality from these two protozoan parasites can be duplicated with rapid oocyst passage and by using enough runs and birds to give a good sampling.

7. Any one run with a group of ten two-week old chicks, Vantress Cross (White Rocks), may produce from 10-70 percent deaths with E. tenella and 0-100 percent deaths with E. necatrix

if 40 thousand sporulated oocysts of the former and 80 thousand sporulated oocysts of the latter are used.

8. E. necatrix is more pathogenic occasionally at the 80 thousand sporulated oocyst level than is E. tenella.

9. At the levels of oocyst inocula used, in mixed infection studies, approximately the same percent mortality resulted regardless of which species was varied or kept constant.

10. The average percent mortality caused by both species in multiple infection was approximately the same as, or greater than, the average percent mortality caused by both species of pure infection at the same inocula levels.

11. At certain inocula levels, mixed infections cause a higher mortality than does pure infections of equal numbers of oocysts.

12. The majority of deaths caused by both pure and mixed infections occurred on the 5th, 6th and 7th days post infection. A few deaths occurred 4 days after infection with pure E. necatrix and eight days post infection with mixed infections.

B. The Effect of Aureomycin on the Sporozoite and Merozoite Stages of E. tenella

1. Aureomycin HCl at the levels of 10 and 20 grams per 50 pounds of feed (.044 and .088 percent respectively) prevent mortality when administered to chickens the day before infection with both E. tenella and E. necatrix.

2. Aureomycin HCl at the .044 percent level did not prevent mortality (E. tenella infection) when administered to the chick the same day as infection.

3. Experiments indicate that Aureomycin HCl at the .022 percent level reduced mortality of chicks infected with E. necatrix when fed to chicks the day before infection.

4. A .044 percent Aureomycin HCl takes between 4 to 8 hours to inactivate sporozoites of E. tenella, while an .088 percent solution will inactivate this stage within 0-2 hours.

5. Sporozoites maintained in a buffered saline solution at 37° C were capable of initiating infection at least 8 hours after removal from the chickens.

6. Under the conditions of these experiments, no infection with the Generation I merozoites occurred.

7. Some evidence indicates that a .088 percent solution of Aureomycin will inactivate Generation II merozoites within 2 hours.

8. In these experiments the survival time of Generation II merozoites maintained in a buffered saline solution at 37° C is somewhere between 2 and 4 hours.

9. Aureomycin at the levels of .044 and .088 percent was capable of reducing or preventing mortality of chickens infected with E. tenella and this protection may have been due, in part, to its activity against the sporozoites and Generation II merozoites.

BIBLIOGRAPHY

Allen, E. A. 1932. The influence of diet on the development of experimental coccidiosis in chickens kept under sanitary conditions. Amer. J. Hyg. 15: 163-185.

Allen, R. W. and Farr, M. M. 1943. Sulfaguanidine as a prophylactic during the period of acquirement of resistance by chickens to cecal coccidiosis. Amer. J. Vet. Res. 4: 50-53.

Anderson, L. P. and Mallman, W. L. 1945. The penetrative powers of disinfectants. Mich. Agri. Exp. Sta. Tech. Bull. 183.

Andrews, Justin. 1933. Effect of oily mixtures of phenolics on the oocysts of poultry coccidia with special reference to E. tenella. J. Parasit. 19: 124.

_____. 1934. Resistance and susceptibility in coccidial infection. J. Parasit. 20: 332.

Babcock, W. E. and Dickinson, E. M. 1954. Coccidial immunity studies in chickens. 2. The dosage of Eimeria tenella and time required for immunity to develop in chickens. Poultry Sci. 33: 596-601.

Baldwin, F. M., Wiswell, O, B., and Jankiewicz, H. A. 1941. Hemorrhage control in Eimeria tenella infected birds when protected by anti-hemorrhagic factors, Vitamin K. Proc. Soc. Exp. Biol. and Med. 48: 278-280.

Beach, J. R. and Corl, J. C. 1925. Studies in the control of avian coccidiosis. Poultry Sci. 4: 83-93.

Becker, E. R. 1934. The nature of immunity in coccidiosis. J. Parasit. 20: 332.

_____. 1952. Protozoa. (In Biester, W. E. and Schwarte, L. H., Diseases of Poultry.) Iowa State Coll. Press, Ames, 1245 pp.

_____, and Crouch, H. B. 1931. Some effects of temperature upon development of the oocyst of coccidia. Proc. Soc. Exp. Biol. Med. 28: 529-530.

Becker, E. R. and Waters, Philip C. 1938. The influence of the ration on mortality from cecal coccidiosis in chickens. Iowa State Coll. J. Sci. 12: 405-414.

_____, Jessen, R. J., Pattilo, W. H. and Doorninck, W. M. Van. 1956. A biometrical study of the oocyst of Eimeria necatrix, a parasite of the common fowl. J. Protozool. 3: 126-131.

Benedetti, L. M. E. de. 1944. Influencia de factores climaticos sobre los ooquistes de Eimeria tenella. Rev. Fac. Agron. y Vet. 10: 543-557.

Boney, W. A. 1948. The efficacy of methyl bromide as a fumigant for the prevention of cecal coccidiosis. Amer. J. Vet. Res. 9: 210-214.

Boughton, Donald C. 1939. Studies on the control of poultry coccidiosis. I. The sporulation of oocysts in various types of litter. Bull. Univ. Georgia 34: 9-14.

Brackett, S. and Bliznick, A. 1952. The relative susceptibility of chickens of different ages to coccidiosis caused by Eimeria necatrix. Poultry Sci. 31: 146-148.

Brotherston, J. G. 1948. The effect of relative dryness on the oocysts of Eimeria tenella and E. bovis. Trans. R. Soc. Trop. Med. Hyg. 42: 10-11.

Champion, Lloyd R. 1950. The inheritance of resistance to cecal coccidiosis in the domestic fowl. Poultry Sci. 29: 753.

_____. 1952. The inheritance of resistance to Eimeria tenella in the domestic fowl. Poultry Sci. 31: 911.

_____. 1954. The inheritance of resistance to cecal coccidiosis in the domestic fowl. Poultry Sci. 33: 670-681.

Chandler, W. L. 1933. On the control of cecal coccidiosis in chickens. Michigan State Coll. Agri. Exp. Sta. Tech. Bull. 127: 1-27.

Clapham, P. A. 1950. On sterilizing land against poultry parasites. J. Helminth. 24: 137-144.

Cole, L. J. and Hadley, P. B. 1908. Blackhead: a coccidial disease of turkeys. Science, N. Y. 27: 994.

Collier, H. B. and Swales, W. E. 1948. On the chemotherapy of caecal coccidiosis (Eimeria tenella) of chickens. VII, A note on the metabolism of caecal epithelium, normal and parasitized. Canad. J. Res. 26D: 77-81.

Cuckler, A. C. and Malanga, C. M. 1955. The effect of nicarbazin on avian coccidia. J. Parasit. 41(suppl.): 19.

Daughtery, Jack W. 1950. Carbohydrate metabolism in chickens infected with Eimeria tenella. J. Parasit. 36(suppl.): 42.

_____, and Herrick, C. A. 1952. Cecal coccidiosis and carbohydrate metabolism in chickens. J. Parasit. 38: 298-304.

Delaplane, John P. and Stuart, Homer O. 1935. The survival of avian coccidia in soil. Poultry Sci. 14: 67-79.

_____. 1953. Nutrition and poultry diseases. Flour and Feed 52: 16-17.

Dukes, H. H. 1955. The physiology of domestic animals. Seventh ed. Comstock Pub. Assoc., Ithaca, New York. 1020 pp.

Eckardt, . 1903. Ueber Coccidiosis intestinalis bein Geflugel. Berl. Tierarztl. Wochenschr. 177-180.

Edgar, S. A. 1954. Effect of temperature on the sporulation of oocysts of the protozoan, Eimeria tenella. Trans. Amer. Micros. Soc. 73: 237-242.

_____. 1955. Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. J. Parasit. 41: 214-216.

_____. 1956. Alabama's stations vaccine for coccidiosis is "practical." Flour and Feed 57: 25-26.

_____, Herrick, C. A. and Fraser, L. A. 1944. Glycogen in the life cycle of the coccidium, Eimeria tenella. Trans. Amer. Micros. Soc. 63: 199-202.

Ellis, C. C. 1938. Studies on the viability of the oocysts of Eimeria tenella, with particular reference to conditions of incubation. Cornell Vet. 28: 267-274.

Fantham, H. B. 1910. The morphology and life-history of Eimeria (coccidium) avium: a sporozoon causing a fatal disease among young grouse. Proc. Zool. Soc. London 11: 672-691.

_____. 1915. Coccidiosis in poultry and game birds. J. Board of Agric. London 21: 10.

Farr, M. M. 1943. Resistance of chickens to cecal coccidiosis. Poultry Sci. 22: 277-286.

_____, and Wehr, E. E. 1949. Survival of Eimeria acervulina and E. tenella and E. maxima oocysts on soil under various field conditions. Ann. N. Y. Acad. Sci. 52: 468-472.

Fish, Frederic. 1931. The effect of physical and chemical agents on the oocysts of Eimeria tenella. Science 73: 292-293.

Gardiner, J. L. 1955. The severity of cecal coccidiosis infection in chickens as related to the age of the host and the number of oocysts ingested. Poultry Sci. 34: 415-420.

_____. 1957. The effect of Aureomycin and low-level sulfamethazine, separately and in combination, on cecal coccidiosis. Poultry Sci. 36: 159-165.

Gerard, P. 1913. Le cycle evolutif d'une nouvelle Coccidie aviarire, Eimeria bracheti (n. sp.). Arch. f. Protistenk. 29: 193-202.

Gill, B. S. 1955. Observations on the relationship of thyroidal activity of chicks and caecal coccidiosis (Eimeria tenella). Indian J. Vet.Sci. and Anim. Husb. 25: 75-87.

_____, and Ray, H. N. 1954a. Glycogen and its probable significance in Eimeria tenella, Railliet and Lucet, 1891. Indian J. Vet. Sci. and Anim. Husb. 24: 223-228.

_____. 1954b. On the occurrence of mucopolysaccharides in Eimeria tenella, Railliet and Lucet, 1891. Indian J. Vet. Sci. and Anim. Husb. 24: 229-237.

_____. 1954c. Phosphatases and their significance in Eimeria tenella, Railliet and Lucet, 1891. Indian J. Vet. Sci. and Anim. Husb. 24: 239-244.

Goldsby, Alice I. and Eveleth, D. F. 1950. Immunizing baby chicks against cecal coccidiosis. North Dakota Agri. Exp. Sta. Bull. No. 361. 12 pp.

Goodrich, H. P. 1944. Coccidian oocysts. Parasitology 36: 72-79.

Gordeuk, S. Jr., Bressler, G. O, and Glantz, P. J. 1951. The effect of age of bird and degree of exposure in the development of immunity to cecal coccidiosis in chicks. Poultry Sci. 30: 503-508.

Greven, U. 1953. Zur Pathologie der Geflugelcoccidiose. Arch. f. Protistenk. 98: 342-411.

Hadley, P. B. 1909. Studies in avian coccidiosis. I. White diarrhea of chicks. II. Roup of fowls. Centralbl. f. Bakteriol. 50: 348-353.

_____. 1911. Eimeria avium: a morphological study. Archiv. f. Protistenk. 23: 7-50.

Hawkins, P. A. 1945. Report of the School of Veterinary Medicine, Michigan State College.

Henry, Dora Priaulx. 1932. The oocyst wall in the genus Eimeria. Univ. of Calif. Publ. Zool. 37: 269-278.

Herrick, C. A. 1933. Some physiological effects of coccidia on chickens. J. Parasit. 19: 123-124.

_____. 1934. The development of resistance to the protozoan parasite, Eimeria tenella, by the chicken. J. Parasit. 20: 329-330.

_____. 1935. The persistence of immunity of the chicken to the protozoan parasite, Eimeria tenella. J. Parasit. 21: 429.

_____. 1950. The effect of coccidia on the metabolism and temperature regulation of chickens. Poultry Sci. 29: 763.

_____, Ott, G. L. and Holmes, C. E. 1936. Age as a factor in the development of resistance of the chicken to the effects of the protozoan parasite, Eimeria tenella. J. Parasit. 22: 264-272.

Horton-Smith, C. 1947. Coccidiosis. Some factors influencing its epidemiology. Vet. Rec. 59: 645.

Horton-Smith, C., Taylor, E. L. and Tuttle, E. E. 1940. Ammonia as a fumigant for coccidial disinfection. Vet. Rec. 52: 829-832.

_____, and Taylor, E. L. 1945. Sulphamethazine in the drinking water as a treatment for cecal coccidiosis in chickens. Vet. Rec. 57: 35-36.

Jankiewicz, H. A. 1942. Symptoms and immunity following graduated doses of Eimeria tenella. J. Parasit. 28 (suppl.): 23.

_____, and Scofield, R. N. 1934. The administration of heated oocysts of Eimeria tenella as a means of establishing resistance and immunity to cecal coccidiosis. J. Amer. Vet. Med. Assoc. 84: 507-526.

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

_____. 1927. Immunity or resistance of the chicken to coccidial infection. Oregon Agri. Exp. Sta. Bull. No. 230. 31 pp.

_____. 1930. Director's Biennial Report for the Fiscal Years 1928-30. Oregon Agri. Exp. Sta., 119-120.

Jones, E. Elizabeth. 1934. The effect of diet on the course of experimental coccidiosis infection in the chicken. J. Amer. Vet. Med. Assoc. 38: 193-206.

Jukes, H. G., Hill, D. C. and Branion, H. D. 1956. Effect of antibiotic on the rate of passage of feed marker through the digestive tract of the chick. Poultry Sci. 35: 232-234.

Kennard, D. C. and Chamberlin, V. D. 1949. Growth and mortality of chicks as affected by the litter. Ann. New York Acad. Sci. 52: 583-588.

Koutz, F. R. 1948. Immunity studies in avian cecal coccidiosis. I. The value of drugs to establish immunity in young chickens. Amer. J. Vet. Res. 9: 388-395.

_____. 1950. The survival of avian coccidia in the soil. The Speculum 3: 1-5.

_____. 1953. The effect of built-up litter on the parasite ova and oocyst of poultry. Second Report. Poultry Sci. 32: 313-320.

Koutz, F. R. 1955. Immunity studies in avian coccidiosis. III. Variations in the development of immunity in compost litter (old built-up litter). Speculum 8: 23-25.

Levine, Lawrence and Herrick, C. A. 1954. The effect of the protozoan parasite Eimeria tenella on the ability of the chicken to do muscular work when its muscles are stimulated directly and indirectly. J. Parasit. 40: 525-531.

_____. 1955. A comparative study of the effects of cecal coccidia on the ability of Plymouth Rocks and Leghorn chickens to do muscular work. J. Parasit. 41(suppl.): 19.

Levine, P. P. 1938. Eimeria hagani n. sp. (Protozoa: Eimeridae) a new coccidium of the chicken. Cornell Vet. 29: 263-266.

_____. 1940. The initiation of avian coccidial infection with merozoites. J. Parasit. 26: 337-343.

_____. 1942a. A new coccidium pathogenic for chickens, Eimeria brunetti n. sp. (Protozoa: Eimeridae). Cornell Vet. 32: 430-439.

_____. 1942b. Excystation of coccidial oocysts of the chicken. J. Parasit. 28: 426-428.

Mayhew, Roy L. 1934a. Studies on coccidiosis. VII. Effects of starvation and removal of ceca. Poultry Sci. 13: 350-369.

_____. 1934b. Studies on coccidiosis. VIII. Immunity or resistance to infection in chickens. J. Amer. Vet. Med. Assoc. 85: 729-734.

_____. 1937. Studies on coccidiosis. X. Histopathology of the caecal type in the chicken. Trans. Amer. Micros. Soc. 56: 431-446.

McDermott, J. J. and Stauber, L. A. 1954. Preparation and agglutination of merozoite suspension of the chicken coccidium, Eimeria tenella. J. Parasit. 40(suppl.): 23

Morgan, B. B. and Hawkins, Philip A. 1952. Veterinary Protozoology. Burgess Publishing Co., Minneapolis, Minn. 187 pp. 23 plates.

Natt, Michael P. and Herrick, Chester A. 1955. The effect of cecal coccidiosis on the blood cells of the domestic fowl. I. A comparison of the changes in the erythrocyte count resulting from hemorrhage in infected and mechanically bled birds. The use of the hemacrit value as an index of the severity of the hemorrhage resulting from infection. Poultry Sci. 34: 1100-1106.

_____. 1956. The effect of cecal coccidiosis on the blood cells of the domestic fowl. II. The changes in the blood volume during the course of the infection. Poultry Sci. 35: 311-316.

Palafox, A. L., Alicata, J. E. and Kartman, L. 1949. Breeding chickens for resistance to cecal coccidiosis. World's Poultry Sci. J. 5: 84-87.

Peterson, F. D. 1933. Studies on the viability of Eimeria tenella in the soil. Cornell Vet. 23: 232-249.

Pattillo, W. H. and Becker, E. R. 1955. Cytochemistry of Eimeria brunetti and E. acervulina of the chicken. J. Morph. 96: 61-96.

Perard, C. 1925. Recherches sur les coccidies et les coccidioses du lapin. Ann. Inst. Pasteur, Paris 39: 505-542.

Pratt, Ivan. 1937. Excystation of the coccidia, Eimeria tenella. J. Parasit. 23: 426-427.

_____. 1940. The effect of Eimeria tenella (Coccidia) upon the blood sugar of the chicken. Trans. Amer. Microsc. Soc. 59: 31-37.

_____. 1941. The effect of Eimeria tenella (Coccidia) upon the glycogen stores of the chickens. Amer. J. Hyg. 34: 54-61.

Railliet, A. and Lucet, A. 1891. Note sur quelques especes de coccidies encore peu etudies. Bull. Soc. Zool. France 16: 246-250.

_____. 1913. Quel. nom doit-on a la coccidie intestinale de la poule? Arch. Paras. 16: 147.

Ray, H. N. and Gill, B. S. 1954. Preliminary observations on akaline phosphatase in experimental Eimeria tenella infection in chicks. Ann. Trop. Med. and Parasit. 48: 8-10.

_____. 1955. Observations on the nucleic acids of Eimeria tenella Railliet and Lucet, 1891. Indian J. Vet. Sci. and Anim. Husb. 25: 17-23.

Riedel, Bernard B. 1950. The effect of ascarid infections on the susceptibility of chickens to coccidiosis. Poultry Sci. 29: 201-203.

Ripson, C. A., Johnson, C. A. and Herrick, C. A. 1949. Some host-parasite relationships between the chicken and its protozoan parasite, Eimeria tenella. Ann. New York Acad. Sci. 52: 473-477.

Rivolta, S. 1878. Delle gregarinosi dei polli e dell' ordinamento delle gregarine e dei psorospermi degli animale domestici. Giorn. Anat. Fisiol. e patol. Animali, Pisa. 10: 220.

Rosenberg, Morton M. 1941. A study of the inheritance of resistance to Eimeria tenella in the domestic fowl. Poultry Sci. 20: 472.

Rosenberg, M. M., Alicata, J. E. and Palafox, A. L. 1954. Further evidence of hereditary resistance and susceptibility to cecal coccidiosis in chickens. Poultry Sci. 33: 972-980.

Schaudinn, T. 1900. Untersuchungen uber generationswechel bei Coccidien. Zool. Jahrb. Anat. 13: 197.

Schildt, C. A. and Herrick, C. A. 1955. The effect of cecal coccidiosis on the motility of the digestive tract of the domestic fowl. J. Parasit. 41(suppl.): 18-19.

Scholtseck, E. 1953. Beitrag zur kenntnis des Entwicklungsganges des Huhnercoccids Eimeria tenella. Arch. f. Protistenk. 98: 415-465.

Seeger, K. C. 1946. Sulfamethazine in the treatment of induced and natural Eimeria tenella infections. Poultry Sci. 25: 411.

_____, Lucas, W. C. and Tomhave, A. E. 1951. The effect of dried crude Aureomycin on cecal coccidiosis in chickens. Poultry Sci. 29: 779.

Skoglund, W. C. 1952. Effect of built-up litter on coccidia and other parasites. World's Poultry Sci. J. 3: 293.

Smetana, Hans . 1933. Coccidiosis of the liver. Arch. Path. 15: 175-192.

Smith, B. F. and Herrick, C. A. 1944. The respiration of the protozoan parasite, Eimeria tenella. J. Parasit. 30: 295-302.

Swales, W. E. 1946. The chemotherapy of cecal coccidiosis (Eimeria tenella) of chickens. J. Amer. Vet. Med. Assoc. 108: 393-400.

Thorp, W. T. S., Gordeuk, S., Glantz, P. J. and Learned, M. 1947. The chemotherapy of cecal coccidiosis. Amer. J. Vet. Res. 8: 196-203.

Todd, A. C. and Hansen, M. F. 1948. Eimeria tenella infection in chickens with induced mild hyperthyrosis. J. Parasit. 34: 29-30.

Todd, A. C., Culton, T. G., Kelley, G. W. and Hansen, M. F. 1949. Induced hyperthyroidism and growth of parasitized chicks. Poultry Sci, 28: 549-551.

Tomhave, A. E. 1949. Effect of litter moisture on outbreaks of coccidiosis. Ann. New York Acad. Sci. 52: 460-463.

Tyzzer, E. E. 1929. Coccidiosis in gallinaceous birds. Amer. J. Hyg. 10: 1-115.

_____, Theiler, H. and Jones, E. E. 1932. Coccidiosis in gallinaceous birds. II. A comparative study of species of Eimeria of the chicken. Amer. J. Hyg. 15: 319-393.

Uricchio, William A. 1953. The feeding of artificially altered oocysts of Eimeria tenella as a means of establishing immunity to cecal coccidiosis in chickens. Proc. Helminth. Soc. Washington 20: 77-83.

Waletzky, E. and Hughes, C. O. 1946. The effectiveness of short treatment with sulfonamides in cecal coccidiosis of the chicken. J. Parasit. 32(suppl.): 9.

_____. 1949. Factors involved in tests for acquired immunity in Eimeria tenella infections of the chicken. Ann. New York Acad. Sci. 52: 478-495.

Warner, David E. 1933. Survival of coccidia of the chicken in soil and on the surface of eggs. Poultry Sci. 12: 343-348.

Waxler, S. H. 1941a. Immunization against cecal coccidiosis in chickens by the use of x-ray attenuated oocysts. J. Amer. Vet. Med. Assoc. 99: 481-485.

_____. 1941b. The effect of feeding concentrated physiological saline to chickens during cecal coccidiosis. Amer. Micros. Soc. Trans. 60: 453-460.

Wheeler, Robert S., Hoffman, Edmund, and Barber, Clifford W. 1948. The effect of thiouracil-induced hyperthyroidism on the resistance of chicks artificially infected with Eimeria tenella. J. Amer. Vet. Med. Assoc. 112: 473-474.

Wickware, A. B. 1949. Studies on experimental Eimeria tenella infection in chickens fed dietary supplements. Canadian J. Comp. Med. 13: 229-237.

Yacowitz, H. and Bird, O. D. 1953. Antibiotic levels in the digestive tract of the chick. Poultry Sci. 32: 966-968.

Young, B. P. 1929. A quantitative study of poultry coccidiosis. J. Parasit. 15: 241-250.

APPENDIX

PLATE I

Figs. 1 - 8. Ceca of chickens infected with *E. tenella*, one through eight days after infection.

Figs. 9 - 16. Small intestines of the same chickens.

PLATE I

CECA AND SMALL INTESTINE OF CHICKS INFECTED WITH *E TENELLA*

(1-8 DAYS AFTER INFECTION)

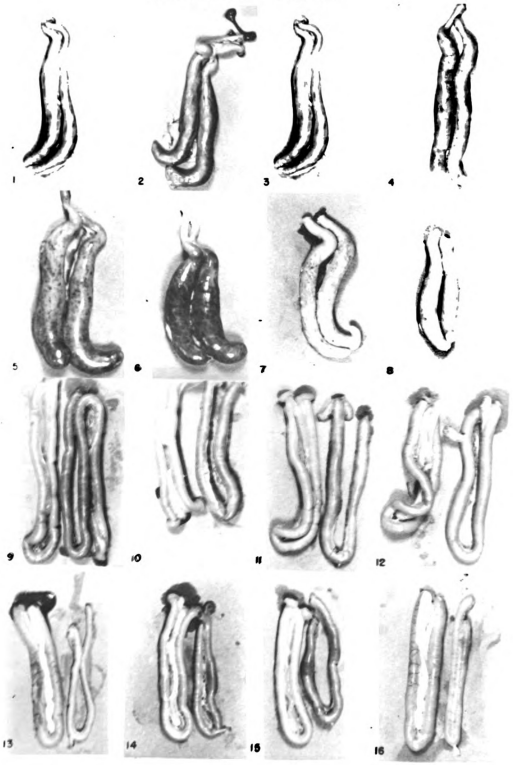


PLATE II

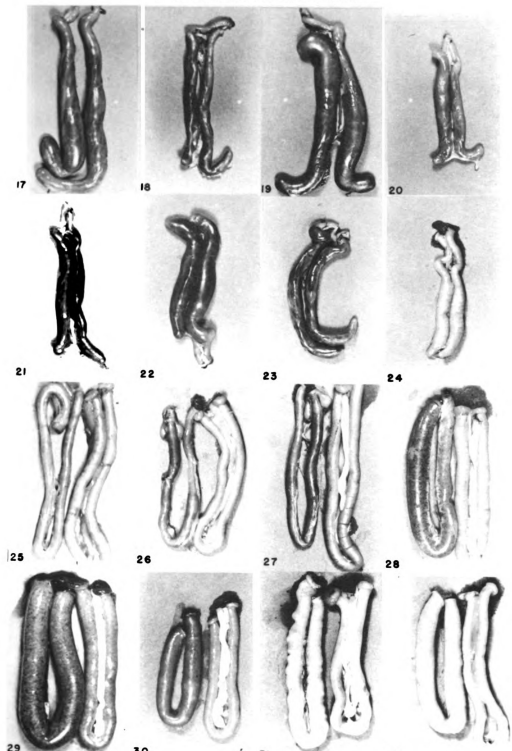
Figs. 17 - 24. Ceca of chickens infected with *E. necatrix*, one through eight days after infection.

Figs. 25 - 32. Small intestines of the same chickens.

PLATE II

CECA AND SMALL INTESTINE OF CHICKS INFECTED WITH *E. NECATRIX*

(1-8 DAYS AFTER INFECTION)



SOME ASPECTS ON CHICK MORTALITY WITH PURE AND MIXED INFECTIONS OF THE PROTOZOAN PARASITES, EIMERIA TENELLA AND EIMERIA NECATRIX AND IN VITRO STUDIES ON THE EFFECT OF AUREOMYCIN ON THE SPOROZOITES AND MEROZOITES OF E. TENELLA

By

William Dunsmore Wilson

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

Year 1957

Approved by William E. Landgraf

This study was undertaken to ascertain: (1) the possibility of making LD_{50} determinations for Eimeria tenella and E. necatrix and duplicating these results, (2) what influence, if any, mixed infection plays on mortality, and (3) the effect of Aureomycin HCl* on cecal coccidiosis mortality, and whether or not any effect would be due to direct action of this antibiotic on the parasite.

Each strain (one each of E. tenella and E. necatrix) was isolated by a single oocyst infection for the so-called "pure line strain" which was used throughout these experiments.

White Rock, Vantress Cross, straight-run chicks were obtained from the same brooder flock the same day as hatching or as day-old chicks and maintained until approximately two weeks of age before inoculation.

Inoculation, oocyst collection and oocyst counting were done utilizing normal methods except in oocyst collection a series of sieves (sieve sizes - National Bureau of Standards Series - of 100, 200 and 325) were used to eliminate extraneous material whose size exceeded approximately 44 u.

Sporozoite and merozoite stages of E. tenella were collected from inoculated chicks and maintained in buffered saline and various percentages (.022, .044 and .088) of Aureomycin in solution for several time periods (1/12 to 16 hours). After each time period, non-infected chicks were inoculated via the cloaca. Collection, maintenance and inoculation were carried out in a walk-in incubator at 37° C.

The conclusions obtained from the results were:

A. Pure and Mixed Infections.

1. Eimeria tenella infection caused a high mortality rate with two-week old Vantress Cross (White Rock) chicks.

2. E. tenella infection caused the maximum average percent of mortality (39 and 41) at the 40 and 60 thousand oocyst levels studied.

3. Eimeria necatrix infection caused a high mortality with similar birds.

4. E. necatrix infection caused the highest percent of mortality at the 80 thousand oocyst inocula level per bird with respect to the 20, 40 and 60 thousand oocyst levels studied.

5. There was some indication that the maximum percent mortality was never realized with the oocyst inocula levels used in the E. necatrix studies.

6. Mortality for these two protozoan parasites can be duplicated with rapid oocysts passage and by using enough "runs" and birds to give a good sampling.

7. Any one "run" with a group of ten, two-week old, chicks, Vantress Cross (White Rock), produced from 10-70 percent deaths with E. tenella and 0-100 percent deaths with E. necatrix if 40 thousand sporulated oocysts of the former and 80 thousand sporulated oocysts of the latter were used.

9. At the levels of oocyst inocula used, in mixed infection studies, approximately the same percent mortality

resulted regardless of which species was varied or kept constant.

10. The average percent mortality caused by both species in multiple infection was approximately the same as, or greater than, the average percent mortality caused by both species of pure infection at the same inocula levels.

11. At certain levels, mixed infections caused a higher mortality than pure infections of equal numbers of oocysts.

12. The majority of deaths caused by both pure and mixed infections occurred on the 5th, 6th and 7th days post infection. A few deaths occurred 4 days after infection with pure E. necatrix and eight days post infection with mixed infections.

B. The Effect of Aureomycin on the Sporozoite and Merozoite Stages of E. tenella.

1. Aureomycin HCl at the levels of 10 and 20 grams per 50 pounds of feed (.044 and .088 percent, respectively) prevented mortality when administered to chickens the day before infection with both E. tenella and E. necatrix.

2. Aureomycin HCl at the .044 percent level did not prevent mortality (E. tenella infection) when administered to the chick the same day as infection.

3. Experiments indicated that Aureomycin HCl at the .022 percent level reduced mortality of chicks infected with E. necatrix when fed to chicks the day before infection.

4. A .044 percent Aureomycin HCl took between 4 to 8 hours to inactivate sporozoites of E. tenella, while an .088 percent solution inactivated this stage within 0-2 hours.

5. Sporozoites maintained in a buffered saline solution at 37° C were capable of initiating infection at least 8 hours after removal from the chickens.

6. Under the conditions of these experiments, no infection with the Generation I merozoites occurred.

7. Some evidence indicated that a .088 percent solution of Aureomycin inactivated Generation II merozoites within 2 hours.

8. In these experiments the survival time of Generation II merozoites maintained in a buffered saline solution at 37° C was somewhere between 2 and 4 hours.

9. Aureomycin at the levels of .044 and .088 percent was capable of reducing or preventing mortality of chickens infected with E. tenella and this protection may have been due, in part, to its activity against the sporozoites and Generation II merozoites.

---

*Aureomycin® HCl = crystalline chlortetracycline HCl.