AN ABSTRACT OF THE THESIS OF

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Title Studies of Immunity to Coccidiosis in Domestic Rabbits

Abstract Approved

This study was undertaken in order to determine the degree of immunity developed by the domestic rabbit to coccidiosis. Five species of coccidia, all of the genus Eimeria, have been isolated and described as occurring in the domestic rabbit. All of these species were found to occur in field outbreaks of coccidiosis in Oregon. Each species was isolated in pure culture and studied separately.

Groups of rabbits were given various sized doses of pure culture of a single species of coccidia, and four weeks later were checked with a second dose of coccidia of the same species. This check was made to determine the degree of immunity that had been developed. Following this check for immunity, individual rabbits known to be immune to one species were given a large dose of another species, in order to determine whether or not the immunity was species specific.

This study was carried out in five trials, using one species for the basic study of each trial.

There are no serological or tissue reaction tests that can be used to determine the degree of immunity of a host against coccidiosis. The correlation of clinical symptoms and the number of organisms in the feces form the bases for estimating the resistance of the host to the disease.

When the same sized dosage was used, the degree of immunity developed by the host to each of the species was about equal. When the dosage exceeded 50,000 oöcysts, sufficient immunity was developed to prevent clinical symptoms when a large subsequent dose was administered. Doses of 25,000 oöcysts developed partial immunity against subsequent doses of the parasites. Rabbits that received 1,000 oöcysts daily for five days appeared to have developed no immunity against the disease.

Rabbits immune to one species of coccidia were susceptible to any of the other species. Based on either the clinical symptoms or the number of oöcysts in the feces, the organisms are strictly species specific.

Regardless of the degree of immunity that the host exhibited against the disease, there was always some increase in the number of oöcysts in the feces following a dose of the parasites. This would indicate that immunity to coccidiosis is not absolute as we generally regard absolute immunity to bacterial and virus diseases.
The symptoms of coccidiosis are not characteristic of the species. There were some variations in the pathogenicity of the species as demonstrated by clinical symptoms, but these variations were in the severity of the disease and not in the manifestations.

Individual rabbits did not demonstrate any variation in their susceptibility to the disease. The older rabbits appeared to be better able to withstand the infection as based on clinical symptoms, but based on the number of oocysts in the feces they were equally susceptible.

The effect of the disease on the body weight of the host was the most constant observation. All rabbits that received sufficient oocysts to develop immunity ate less feed and lost weight during the acute stages of the disease. This observation must be the basic consideration in considering the importance of the control of the disease.

The rabbit industry is dependent upon being able to market rabbits at the frying weight within ten weeks. Any interruption of the program will seriously affect the profits of the industry; thus a control program must be developed that prevents infection during the first ten weeks of the rabbit's life.
STUDIES OF IMMUNITY TO COCCIDIOSIS IN DOMESTIC RABBITS

by

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A THESIS

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STUDIES OF IMMUNITY TO COCCIDIOSIS
IN DOMESTIC RABBITS

I. INTRODUCTION

Coccidiosis of domestic rabbits is a parasitic disease that may
develop either in the epithelium of the bile ducts of the liver or
in the epithelium of the small intestine, depending upon the species
of the organism present.

It is the most widely spread and serious disease of the rabbit
industry in the State of Oregon. The industry is not well enough
organized to be able to obtain the statistical data that is required
to make the proper appraisal of the economic importance of coccidiosis,
but from our own observations and correspondence, the control or lack
of control of this disease is often the determining factor of success
or failure of a commercial rabbitry.

During the period of time covered by this study, post-mortem and
fecal examinations of rabbits sent to the laboratory of the Department
of Veterinary Medicine, Oregon State College, revealed that 89 per cent
of the examinations were positive for coccidia.

Numerous field trips were made in conjunction with these studies,
in order to observe the conditions under which outbreaks of
coccidiosis had occurred. It was found that the majority of the
rabbitries were infected with coccidia. The most serious losses
occurred where the species of the organism that develop in the small
intestine predominated. It was also observed that husbandry and
hutch construction had a more direct relationship to the extent of
the severity of the disease than did the species of the organism.

These preliminary studies indicated that the problems of coccidiosis confronting the rabbit industry were much the same as those of the poultry industry; thus the control of coccidiosis through the development of immunity, that has been done so successfully by the poultry industry, was used as the basis of this study.

II. ETIOLOGY

In the domestic rabbit, *Oryctolagus cuniculus* (Linnaeus), five species of coccidia, all of the genus *Eimeria*, have been found to occur in natural infections (16, p. 10).

Prior to the work published by Kessel (14, p. 100), and Kessel and Jankiewicz (15, p. 304), there was much confusion as to the species of coccidia found in the domestic rabbit. It had been assumed that there were only two species, *Eimeria stiedae* and *Eimeria perforans*. In their research, these men made careful studies of many varieties of coccidia occurring in natural infections, and observed a wide variation in morphology, physiology, and life cycles. This study led to the establishment of the five species as they are known today. They stated (15, p. 304) that Perard, working independently, had found and published in 1925 a new species, *Eimeria magna*, Perard. Perard's work was confirmed and this species was included with *Eimeria media*, Kessel 1929, and *Eimeria irresidua*, Kessel and Jankiewicz 1935. The bases of identification set forth by Kessel and Jankiewicz (15, p. 307) depend primarily on the study
of the sporulated oocyst, as they did not make an extensive study of the histopathology associated with the endogenous phase of the life cycle.

Rutherford (16, p. 27) made an exhaustive study of the endogenous cycle of the species of coccidia found in the intestinal epithelium of domestic rabbits. He found that each of the species named by Kessel and Jankiewicz could be identified by morphological studies of their endogenous forms, and in this manner showed them to be true species and not varieties of the same species.

Species identification as given by Kessel and Jankiewicz (15, p. 324) and confirmed by Rutherford (16, p. 27) were used in the isolation and identification of pure cultures in this study. Careful attention was given to all material, so that no new species would go unrecognized.

Coccidia belonging to the genus *Eimeria* are characterized by the production of more or less spherical, ellipsoidal, or ovoid oocysts, which when sporulated contain four sporocysts, each of which contains two sporozoites (21, p. 829). The species vary in their shape, size, color, micropyle, and the presence or absence of a residual body in the sporulated oocyst and sporocyst. Other characteristics that are used for species identification are the length of time required for the sporulation of the oocyst and the completion of the endogenous cycle.

The species belonging to the genus *Eimeria* are with very few exceptions strictly host specific (7, p. 151). In the few cases
where they are not strictly host specific, they will develop only in a host that is closely related to the natural host (19, p. 363).

III. LIFE CYCLE

The life cycles of the five species of coccidia found in the domestic rabbit are very similar. The principal differences are the length of time required for the sporulation of the oöcyst and the development of the endogenous cycle. Due to this similarity, only the life cycle of the species *Eimeria irresidua* will be outlined.

There are two major divisions of the life cycle of coccidia belonging to the genus *Eimeria*. The exogenous cycle takes place outside the epithelial cells of the host and includes the fertilization of the oöcyst, its sporulation, and subsequent liberation of the sporozoites. The endogenous cycle takes place within the epithelial cell of the host; beginning with the sporozoite entering the cell, then the development of the first generation schizont, gametogenesis, and the liberation of the microgametocyte and macrogametocyte.

As a matter of convenience, a description of the life cycle may commence with the exogenous cycle. The macrogametocyte, upon liberation from the epithelial cell, is the infertile oöcyst. Fertilization takes place by a microgametocyte entering the micropylor end of a macrogametocyte. The oöcyst then passes from the body of the host through the intestinal tract. At this time the organism is not infectious and appears as an ovoidal cell, consisting
of a definite cell wall and a large central nucleus. One end of the organism is slightly constricted and bears a micropyle.

Under favorable conditions of temperature, moisture, and oxygen the nucleus changes form, first by decreasing in size and then dividing into four, roughly-outlined masses. These masses or sporocysts elongate and divide within themselves into elongated bodies, the sporozoites. In the Eimeria irresidua there is no residual body formed during the division of the nucleus into the sporocysts, but there is a small residual body within the sporocyst when the sporozoites are formed. The complete sporulation of the Eimeria irresidua requires at least 50 hours at a temperature of 36° centigrade. At temperatures below this reading, the length of time required for sporulation is proportionately increased.

Sporulation ceases entirely at about 10° centigrade.

The only manner of infection is the ingestion of the sporulated oocyst by a susceptible host. This may come about by the contamination of the food or water, or by eating contaminated bedding. Within a few hours after the oocysts are ingested, the sporozoites can be found free in the lumen of the intestines. The excystation of the oocyst takes place in the fore part of the duodenum in the presence of pancreatic juice (2, p. 27). The sporozoite may enter the epithelial cells at once, or may remain in the lumen of the small intestine for three or four days before entering a cell (16, p. 13).
The study of the endogenous cycle of the genus *Eimeria* has been developed over a long period of time and much confusion and speculation had resulted until the investigations of Rutherford were published in 1943 (16, p. 30). The histological studies by this author clarified many of the existing uncertainties. The following description of the endogenous cycle of *Eimeria irresidua* is based entirely on Rutherford's publication.

The liberated sporozoites enter the epithelial cells of the small intestine and develop the first generation of schizonts. Rutherford (16, p. 29) has clearly pointed out that there are types A and types B schizonts formed in this generation and that they can be distinguished by their form, location in the cell, and the morphology of the merozoites that they develop. Both types A and B first generation schizonts complete their development and liberate their merozoites on the sixth day. These liberated merozoites are now free to re-enter an epithelial cell and develop a second generation schizont or gamete. The type A first generation merozoite forms a type A second generation schizont or gamete that develops the microgametocyte. The type B first generation merozoite forms a type B second generation schizont or gamete, resulting in the development of the macrogametocyte or oöcyst. Two days are required to complete the second generation. Late on the ninth day or early on the tenth day, following the ingestion of the sporulated oöcysts, the oöcysts will appear in the fecal material of the host.
By determining the two types of schizonts developed from the sporozoites and tracing their subsequent development through the second generation, Rutherford (16, p. 31) has shown that the entire endogenous cycle is sexual.

IV. MATERIALS AND METHODS

All of the rabbits used in this study were raised in the experimental animal room of the Department of Veterinary Medicine. The breeding stock consisted of Standard New Zealand white rabbits, and only coccidia-free animals were used.

The breeding pens were self-cleaning, and all had hardware cloth floors. Beneath the pens were metal trays to collect all excreta and spilled feed. Standard, glazed, earthenware crocks were used for feed and water. The nesting boxes consisted of apple boxes that were supplied with straw bedding.

Throughout the experiment, the basic diet consisted of commercial rabbit pellets that were purchased in large amounts, so that there would be no interruption of the feeding schedule. The basic diet was supplemented with rolled whole barley, green feed, and root stock. The green feeds consisted of grass clippings in the summer and kale in the winter. The root stock consisted of carrots. The green feed and root stock were fed semi-weekly in amounts that would be eaten in one day. All excess was removed from the pens the same day.
Just prior to kindling and throughout the lactation period, the doe received whole barley. This grain was kept before the young rabbits as soon as they came out of the nest box, so that they would become accustomed to dry feed as soon as possible. This practice facilitates weaning at an early age.

The rabbits were weaned at about four weeks and the doe removed from the pen. The young were checked for coccidia and given two weeks to become adjusted to the change in diet. At about six weeks of age, the rabbits were considered suitable for the trials. Most of our rabbits averaged about one kilogram in weight at six weeks, and the controls gained about one kilogram during the next four or five weeks.

The coccidia used in this study were pure cultures, isolated from field cases of coccidiosis. During routine autopsies in the laboratory, the feces and bile of all rabbits were examined for coccidia and, in all the positive cases, the fecal material was sporulated and the species determined by microscopic examination of the sporulated oöcysts.

The majority of the cases showed mixed infections, but two rabbits were found to be infected with a single species of coccidia, *Eimeria irresidua* and *Eimeria magna*. Very few rabbits were infected with *Eimeria stiedae* and, in each of these cases, one or more species of the coccidia that inhabit the intestine were also found. Pure culture of *Eimeria stiedae* can easily be isolated by collecting the oöcysts from the lesions of the liver and the contents of the gall
bladder.

The following procedure was used in collecting and sporulating the oocysts:

1. Fecal material from live rabbits or the cecal contents of dead animals was placed in large petri dishes and thoroughly moistened with a two per cent solution of potassium dichromate.

2. A clip was placed on the flange of the bottom half of the petri dish. Then the cover was placed in position. This was to allow the free passage of air to the surface of the material, as well as to prevent excessive evaporation.

3. The material was kept at room temperature and stirred twice daily, in order to assure sufficient oxygen for maximum sporulation. As the material lost moisture, distilled water was added to replace the loss.

4. Microscopic examinations were made daily until sporulation was 85 to 90 per cent complete.

5. The material was then washed through a series of five sieves, commencing with one having meshes measuring 1080 micron and finishing with one measuring 149 microns.

6. The washings were concentrated by centrifuging and the supernatant fluid decanted. The specific gravity of the coccidia is about 1.5; therefore they can easily be separated from an aqueous suspension.

7. A concentrated solution of sodium chloride was added to the residue containing the oocysts. By centrifuging, the organisms rose
to the top of the tubes and were decanted.

8. To remove the excess sodium chloride, the oöcysts were diluted with distilled water and concentrated as in step six. Usually this was repeated two or three times.

9. Microscopic examinations were made to determine the species of oöcysts and the percentage of sporulation of the oöcysts.

10. A two per cent solution of sodium dichromate was added to the oöcysts and they were placed in the ice box for storage.

The above procedure was used for all materials sporulated, regardless of whether it was merely for species identification or the isolation of pure cultures.

Two methods were used to count the oöcysts in suspension. One method was to make a count using the standard haemacytometer. The second method was to make a direct count of a given volume of the suspension, by floating the oöcysts on a saturated solution of sodium dichromate in a counting cell. The first method was used most commonly and the averages of serial dilutions taken as the count. After the total count was determined, it was corrected so that the final count represented only sporulated oöcysts.

The number of sporulated oöcysts in each cubic centimeter of the stock cultures was thus determined. When a dosage was chosen, the stock culture suspension was thoroughly mixed and the volume, representing the desired number of oöcysts, was drawn off with a pipet. This suspension was washed several times with distilled water, in order to remove the excess potassium dichromate. Upon the
final washing, the oöcysts were concentrated to a volume less than five cubic centimeters. The dosage was then drawn into a glass syringe and introduced directly into the stomach of the rabbit, through a small rubber tube that was attached to the syringe. The rubber tube should be passed through the esophagus before attaching it to the syringe. A small amount of water was then passed through the syringe and tube to wash the oöcysts from the equipment.

Two methods are used to determine the degree of immunity of an animal to coccidiosis. These are clinical symptoms and the number of oöcysts in the feces following a dose of virulent oöcysts. A correlation of these two manifestations is more satisfactory than attempting to use either one by itself.

Clinical symptoms are observations; thus they are subject to variations. In this study three general terms are used to designate the severity of the disease.

Symptoms classified as mild are those that would likely go unnoticed by the inexperienced observer. Usually there was a decrease in the amount of food consumed and a retarded growth rate. The feces were formed but soft with no evidence of diarrhea. There was always increase in the number of oöcysts in the feces.

Severe symptoms resulted in the total cessation of food consumption, severe diarrhea, dullness, cold cyanotic ears, and sub-normal body temperature. Oöcysts were very numerous in the feces.

Moderate clinical symptoms were those between the two extremes. In these cases, food consumption was continued but decreased, the
rabbits developed diarrhea, and the body temperature remained normal. Oöcysts in the feces were increased but were not as numerous as in the severe cases of the disease.

None of the various species of coccidia studied developed clinical symptoms that were characteristic of the species. It can also be stated that the symptoms may resemble other diseases of rabbits; thus making it necessary to find the parasites before a positive diagnosis can be made.

The second method of determining immunity is by estimating the number of oöcysts in the feces, following a known dosage of virulent organisms. This estimation was made by counting the number of oöcysts in a weighed sample of feces. A wide field microscope was used for this purpose. The magnification used was 100 power and the field of focus had a diameter of two millimeters. Total counts were not practical, so the average of four fields was used.

Salt flotation of a 250 milligram sample of feces was made in a 50 millimeter Syracuse watch glass. After observing the entire surface for uniformity of the distribution of the oöcysts, four fields were counted and the average taken. The following method was used to record the relative number of oöcysts in the feces:

0  total absence of oöcysts
+  five or less oöcysts per field
++ more than five but less than fifteen oöcysts per field
+++ more than fifteen but less than fifty oöcysts per field
++++ more than fifty oöcysts per field.
V. Eimeria irresidua, TRIAL ONE

Eight rabbits, each six weeks old and weighing about one kilogram, were used in this trial. They were divided into pairs and placed into separate pens.

The procedure outlined in this trial is outlined in Table One, page 14. At six weeks of age all rabbits except those in pen one received their initial dose of coccidia. At ten weeks of age all rabbits received a dose of 100,000 oöcysts, in order to determine the degree of immunity that had been developed in pens two, three, and four as well as the susceptibility of the controls in pen one. At fourteen weeks of age cross-immunity and susceptibility tests were made.

The clinical symptoms and relative number of oöcysts in the feces are also recorded in Table One. The weight records of the rabbits from the sixth to the fourteenth week are recorded on Chart One, page 15.
<table>
<thead>
<tr>
<th>Pen number</th>
<th>Rabbit number</th>
<th>Number of oocysts fed at 6 weeks</th>
<th>Eimeria irrisidua</th>
<th>Comparative number of oocysts in feces</th>
<th>Clinical symptoms</th>
<th>Number of oocysts fed at 10 weeks</th>
<th>Eimeria irrisidua</th>
<th>Comparative number of oocysts in feces</th>
<th>Clinical symptoms</th>
<th>Number of oocysts fed at 14 weeks</th>
<th>Cross immunity test</th>
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<th>Clinical symptoms</th>
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<td>E. irrisidua</td>
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<td>E. magna</td>
<td>+</td>
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<tr>
<td></td>
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<td>100,000</td>
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<tr>
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<td>100,000</td>
<td>mild</td>
<td>100,000</td>
<td>E. media</td>
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<td></td>
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<td>5</td>
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<td></td>
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<td>none</td>
<td>100,000</td>
<td>E. perforans</td>
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<td>none</td>
<td>100,000</td>
<td>E. stiedae</td>
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<td>mild</td>
<td></td>
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</tbody>
</table>
VI. *Eimeria magna*, TRIAL TWO

This trial, using *Eimeria magna*, was conducted in exactly the same manner as Trial One. The procedures and results of this trial are recorded on Table Two, page 17.

The weight recordings of the rabbits used in this trial were nearly identical with those in Trial One; therefore no separate chart is necessary to show the effect of this species on the growth rate.
<table>
<thead>
<tr>
<th>Pen number</th>
<th>Rabbit number</th>
<th>Number of oocysts fed at 6 weeks</th>
<th>Eimeria magna</th>
<th>Comparative number of oocysts in the feces</th>
<th>Clinical symptoms</th>
<th>Number of oocysts fed at 10 weeks</th>
<th>Eimeria magna</th>
<th>Comparative number of oocysts in the feces</th>
<th>Clinical symptoms</th>
<th>Arm, summary of selected types, part 1</th>
<th>Comparative number of oocysts fed after 14 weeks</th>
<th>Clinical symptoms</th>
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<td>+</td>
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<td>none</td>
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<td>E. irresidua</td>
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<td>100,000</td>
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<td>E. media</td>
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<tr>
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<td>E. stiedae</td>
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</table>
VII. *Eimeria media*, TRIAL THREE

The procedure followed in this trial was varied somewhat from the preceding trial. The control pen was eliminated and a small daily dose substituted. The procedure and results are recorded on Table Three, page 19. The effects of this species on the growth rate of the rabbits followed the same pattern as the preceding species, but the losses were not as large.

Immunity and susceptibility tests were conducted at ten weeks and cross-immunity tests at the fourteenth week.
<table>
<thead>
<tr>
<th>Pen number</th>
<th>Rabbit number</th>
<th>Number of oocysts fed at 6 weeks, E. media</th>
<th>Comparative number of oocysts in feces</th>
<th>Clinical symptoms</th>
<th>Number of oocysts fed at 14 weeks, E. media</th>
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<th>Cross-immunity test</th>
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VIII. *Eimeria perforans*, TRIAL FOUR

The procedure followed in this trial was the same as trial three, except for slight variations in the cross-immunity checks at the fourteenth week. These variations and the results are recorded in Table Four, page 21. The weight recordings in this trial were nearly identical to those in trial three and followed the same pattern as outlined on Chart One, page 15.
<table>
<thead>
<tr>
<th>Pen number</th>
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<th>E. perfrans fed daily for 6 days</th>
<th>E. perfrans number of oocysts in feces</th>
<th>Clinical symptoms</th>
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<td>died</td>
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<td>E. magna</td>
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<td>★★★ moderate</td>
<td>E. stiedae</td>
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</table>
IX. *Eimeria stiedae*, TRIAL FIVE

Only four rabbits were used in this trial; two of which received 25,000 oöcysts and two received 50,000 oöcysts. At the tenth week each received 100,000 oöcysts in order to check the degree of immunity that had been developed. At fourteen weeks each rabbit received 100,000 oöcysts of one of the other species being studied.
## Table Number 5

<table>
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<th>Pen number</th>
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<th>Number of oocysts fed at 6 weeks, E. stiedae</th>
<th>Comparative number of oocysts in feces</th>
<th>Clinical symptoms</th>
<th>Number of oocysts fed at 10 weeks, E. stiedae</th>
<th>Comparative number of oocysts in feces</th>
<th>Clinical symptoms</th>
<th>Number of oocysts fed at 14 weeks, Cross-immunity test</th>
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<td></td>
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X. DISCUSSION

The present knowledge of coccidiosis has resulted from the development of four basic factors. These factors in the order of their development are host-specificity, multiplicity of species within a single host, development of immunity, and methods of control.

The first major contribution to the present understanding of coccidiosis was the discovery of the strict host-specificity of the parasite. Within the genus *Eimeria*, this restriction is generally regarded as being nearly absolute (7, p. 151).

Many parasitologists had noticed a wide variation in the morphology of the oöcysts found in different animals and birds. While many attempts had been made to classify them, it was generally accepted that they were only varieties of a single species. Dobell (9, p. 193), 1919 in his review of coccidiosis in man, showed that the parasites found in this host could not be established in the dog, cat, rabbit, or any of the other domestic or laboratory animals. Dobell therefore established the foundation for further study in host-specificity and multiplicity of species within a single host.

Before 1923, most papers reviewing the subject of coccidiosis of birds considered but one species. Several varieties were described but little attention was given to host-specificity. Where careful examination had been carried out, it was found that coccidia occurred...
in all species of birds. Oocysts were so frequently found in the English sparrow, *Passer domesticus domesticus* (Linnaeus), that this bird was considered the natural reservoir of the coccidia found in fowls. This assertion was first challenged by Johnson (10, p. 147) where, by carefully controlled experiments, he was unable to infect chickens with coccidia found in the sparrow. He also (10, p. 147) pointed out that the coccidia commonly found in the sparrow belonged to genus *Isopora*, while all the coccidia of the chickens were of the genus *Eimeria*. During the same studies, Johnson was unable to transmit coccidia taken from the chicken to the domestic turkey, *Meleagris mexicana* (10, p. 148). These reports were confirmed by Tyzzer (18, p. 215).

Classification of the species of coccidiosis found in rabbits was equally confusing. Saunders (17, p. 437) published in 1919 a discussion of coccidiosis of rabbits in which he considered only one species, *Eimeria cuniculi*. He stated that this parasite caused infection of the liver, small intestine, and nasal passage. Most investigators up to this time had ignored the fact that Leukart had in 1879 (21, p. 831) pointed out that the coccidium of the liver was a different species from the one he found in the small intestine.

The recognition of the multiplicity of species found within a single host was the second important development in the study of coccidiosis. Pure cultures of the various species of the parasites were isolated and studied separately. Dobell (9, p. 133) found two
species in man. Johnson (10, p. 146) in 1923 questioned the presence of a single species in chickens. Tyzzer (19, p. 383) published on the species of coccidia in chickens in 1929. At that time, he had isolated and studied four species in pure culture. Johnson, in studying cross-immunity tests, added two new species (12, p. 31).

The development of the etiology of coccidiosis of rabbits was summed up by Kessel and Jankiewicz (15, p. 321) and has been discussed under etiology.

Studies of immunity developed largely as the result of observations made during the course of other studies. Pathologists and parasitologists, studying coccidiosis of domestic and laboratory animals, observed that animals recovering from an attack of the disease frequently suffered from a subsequent infection. These observations were the basis of the belief that the host did not develop a resistance to the parasite. The observations were not consistent, and possibilities of at least partial immunity were considered by several investigators about the same time.

Beach and Corl (5, p. 93) noticed that chicks recovering from coccidiosis were resistant to reinfection. Johnson (12, p. 26) had made this same observation, and carrying out controlled experiments showed that a high degree of resistance could be produced by controlled infection of cage-raised birds. Likewise, a high degree of susceptibility could be maintained in birds in which infection had been prevented. Johnson (12, p. 30) also pointed out that there was no reciprocal relationship between the resistance produced by the
coccidia found naturally in the small intestine and those found in the cecum. This was the first indication by controlled experimentation that immunity could be developed and that there was no cross immunity between two species of coccidia. The observations made by Johnson were confirmed by Tyszer (19, p. 380). It was during this study that Tyszer found that several species of coccidia developed in the small intestine of the fowl.

The nature of the immunity developed against coccidia could not be demonstrated by any of the then known serological tests. Backman (3, p. 640), using rabbits, made serological studies in experimental coccidiosis. In this report he considered only two species of the parasites, *Eimeria stiedae* and *Eimeria perforans*. He made no further attempt to classify the intestinal forms. Since this infection was established in his rabbitry, it is likely that no new species of intestinal coccidia were introduced throughout his studies. The results of his experiments showed that none of the known serological tests were of any value in determining the presence of the disease or the degree of immunity.

In a subsequent publication (4, p. 645) based on immunity studies in coccidiosis of rabbits, Backman pointed out that the rabbits, reared in the colony in which previous infection with *Eimeria perforans* had existed, were resistant to experimental infection with the same organisms. These same rabbits were susceptible to infection of *Eimeria stiedae*, regardless of age (4, p. 648).

All attempts failed to produce active or passive immunity from
an antigen, made from the oöcysts or by serum from recovered animals (4, p. 648). This was the first comprehensive effort to study immunity to coccidiosis in rabbits.

Methods were developed for the control of coccidiosis after Johnson's publication (11, p. 23) in 1927. In this report two basic factors were brought out. First that the severity of the disease depended upon the number of oöcysts ingested, and second that the disease followed a limited course. He also pointed out that the fowls completely eliminated the parasites within a month following infection.

On the basis of these facts, the disease was controlled in the poultry flocks by allowing the birds to acquire a natural infection, then by strict sanitary procedures to prevent reinfection until immunity could be developed. The protection prevented acute out-breaks of the disease when the hens reached the age for egg production. In self-cleaning brooders the disease was easily controlled by preventing the contamination of food and water by oöcysts in the feces.

The rabbit industry operates in a somewhat different manner. The majority of the rabbits are marketed at about two months of age; thus any interruption in the growth rate will seriously affect the cost of production. It has been shown in this study that any dosage of oöcysts large enough to cause clinical symptoms would retard the growth rate for about two weeks. It is necessary then to prevent infection in the rabbits that are to be marketed as fryers.
The most successful procedure to follow in preventing the disease is to use self-cleaning hutches and feed containers that can easily be cleaned. As soon as the young rabbits leave the nest box and begin to eat solid food, they are liable to infection. It is at this period that sanitary procedures must be strictly followed. The most satisfactory material for the construction of the hutch floor is 5/8-inch mesh, 19-gauge, galvanized, hardware cloth. The corners and any cross members must be so constructed as to prevent any accumulation of fecal material. There are many types of feeders and watering vessels. Any are satisfactory if they are constructed so that they can be easily cleaned.

The control of coccidiosis by drug therapy is now being studied. The sulfanamides have shown some promise of success, but at this time the information available is not sufficient to warrant the appraisal of their value.

XI. RESULTS

The disease produced by the various species of coccidia in the domestic rabbit is similar in all respects. Due to these similarities, the results of the trials will be discussed as a whole rather than separately. The number of rabbits used in this study was small because of the difficulty in raising and maintaining disease-free rabbits.

The basis of this study was immunity. The other observations, even though equally interesting, were given secondary consideration.
The trials were organized so that each species could be studied separately. After specific immunity had been developed against each species of coccidia, cross-immunity tests were carried out in order to determine whether or not the immunity was species specific.

It was observed with each species that a dosage of 50,000 oocysts or more developed sufficient immunity to protect the host against subsequent infection. Rabbits receiving 25,000 oocysts, either as a single dose or 5,000 oocysts daily for a five-day period, developed partial immunity to the disease. These rabbits were given a larger dose four weeks later and they developed the disease, showing mild clinical symptoms and a moderate increase in the number of oocysts in the feces.

In trials three and four the control pens were eliminated. In substitution of the control pens smaller dosages were given than were used in trials one and two. These rabbits received a daily dose of 1,000 oocysts for a five-day period. All of the rabbits became infected as based on the number of oocysts in the feces, but none of them developed clinical symptoms. Four weeks later when these rabbits received a dose of 100,000 oocysts, they developed clinical symptoms and there was a large increase in the number of oocysts in the feces. No apparent immunity had been developed by this small dosage.

Cross-immunity tests were carried out at fourteen weeks. One-half of the rabbits received a large dose of the species against which they had been immunized. Of the other half, each rabbit
received a dose of one of the other species used in this study. There was no indication in any of the trials that any cross immunity had been developed. This species specificity has been noticed in other animals and reported by Johnson (13, p. 32), Tyzzer, Theiller, and Jones (20, p. 377), Becker (6, p. 13), Dobell (9, p. 193), Andrews (1, p. 185) and others.

Regardless of the degree of immunity developed, there was always an increase in the number of oocysts in the feces following subsequent administration of organisms. Dickinson (8, p. 424) made this same observation in his studies with chickens. The absence of clinical symptoms therefore cannot be used alone as the basis of immunity.

The most characteristic symptoms of coccidiosis are the loss of appetite, loss of body weight, and diarrhea. Other symptoms that were frequently seen were roughened hair coat, drooping of the ears, grinding of the teeth, bloat, mucoid enteritis, cyanotic mucous membranes, and subnormal rectal temperature. The terminal symptoms in fatal cases were prostration, coma, and convulsions.

These trials showed that the severity of the disease depended upon the number and species of oocysts used. Based on clinical symptoms and loss of body weight, Eimeria irresidua and Eimeria magna are more pathogenic than the other species. Rutherford (16, p. 29) made this same observation and suggested that the reason might be due to the fact that in these two species the schizont develops more deeply in the epithelial cells and results in more tissue damage.
Individual rabbits did not demonstrate any noticeable variation in their susceptibility to coccidiosis. Clinical symptoms in rabbits at ten and fourteen weeks were not as severe as those observed at six weeks; however, judging from the number of oöcysts in the feces, they were equally susceptible to infection.

The regularity of the elimination of the oöcysts depends upon the severity of the disease. Where the smaller dosages were used the elimination was regular and followed a definite pattern. On the day following the completion of the endogenous cycle, the oöcysts appeared in the feces in large numbers. The number remained large for about five or six days after which there was a sharp decline. Following this decline oöcysts appeared in the feces for several weeks. Some of the rabbits were held for observation for several months. Where reinfection was prevented, it required at least seven weeks for the complete elimination of the oöcysts. Where reinfection was permitted, oöcysts appeared in the feces for as long as ten months. In severe cases of coccidiosis where profuse diarrhea occurred, the elimination of the oöcysts was sporadic and the number of oöcysts in the feces varied from day to day. It was also noticed that the percentage of fertilized oöcysts decreased in severe cases.

The effect of the disease on the body weight of the host was one of the most constant symptoms. The decrease in the consumption of food very closely correlated with the loss in weight. All rabbits lost weight that received a dose of oöcysts large enough to develop any degree of immunity. The rabbits were weighed twice weekly from
the sixth to the fourteenth week. The chart on page 15 represents the rabbits used in trial one, using *Eimeria irresidua*. The weight pattern represented in this chart is characteristic of the other species. Due to the very close correlation of the five species, only the one chart is necessary to designate the effects of the disease on the growth weight of young rabbits. The results of these trials and field observations indicate that the retarded growth rate caused by the disease is the most serious problem confronting the rabbit industry.
BIBLIOGRAPHY


