A study of bovine coccidiosis

Irl Donaker Wilson

Iowa State College

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A STUDY OF BOVINE COCCIDIOSIS

BY

Irl Donaker Wilson

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject - Veterinary Pathology

APPROVED:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1930
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I. INTRODUCTION

During the past ten years evidence has gradually accumulated showing the widespread occurrence of bovine coccidiosis. It is probable that the disease is nearly universal in distribution in the United States. It is true that in many sections of the country fatalities have not been reported. However, this is no criterion of the economic loss caused by the organism. As in most parasitic diseases, the greatest losses are due to lack of thrift and low growth rates of the parasitized animal. These are apt to pass unnoticed or receive scant attention. Furthermore, in some sections of the country, notably in southwestern Virginia and the adjoining states the disease is so common that records of it have never reached scientific literature. In parts of Virginia coccidiosis of young cattle is so prevalent that it is almost a limiting factor in the beef cattle industry. It exists on almost every farm and fatalities are annual occurrences.

The morphological characteristics of three species of the genus Eimeria, parasitic in cattle, have been well described. However, there are doubtless other species yet to be reported. Little is known of the physiologic characteristics of any of the coccidia and particularly of the species found in cattle. The pathologic changes produced have received some attention but there are many questions yet unanswered.
II. REVIEW OF LITERATURE

A. Historical

The discovery of coccidia is usually credited to Thomas Gordon Hake, an English physician and poet, in 1859. However, in the opinion of Dobell (1922) this distinction belongs to Antony van Leeuwenhoek — the Father of Protozoology. In Leeuwenhoek's unpublished seventh letter dated October 19, 1674, addressed to Henry Oldenburg — then secretary of the Royal Society — and translated by Dobell, he states in part:

"Further, I examined the bile from three old rabbits. The first contained a very few small globules, but very many oval corpuscles of a figure like those that, as I have said, I saw in the bile of an ox. In the bile of the two other rabbits there was nothing but globules, and irregular particles composed of globules joined together; though the thin matter of one was much thicker and more viscid than that of the other, and there were some little clouds floating through it."

In the opinion of Dobell, the oval corpuscles mentioned as being seen "in the bile of the ox" were undoubtedly ova of Fasciola hepatica or a similar trematode while those in the bile of the rabbit were doubtless the oocysts of Eimeria stiedae.

Although Leeuwenhoek did not recognize what he saw, it seems reasonable that he should be given credit for being the
first to discover coccidia. Thus, it follows that the coccidia were among the first of the protozoa to be observed by man.

The first published record of a description of coccidia was by Hake in 1838 who gave description, accompanied by figures, of the unsegmented oocysts of *Eimeria stiedae* in the liver of the rabbit. He refers to the lesions as "carcinoma of the bile ducts" and to the oocysts as "a new form of pus globule." According to Dobell (1922) the lesions produced in the liver of the rabbit were first described and figured by Robert Carswell in 1838 who was at that time Professor of Pathological Anatoay at University College, London. He described the lesions as "a beautiful illustration of the seat of tuberculous matter in the liver of the rabbit." In the opinion of Dobell the lesions are clearly those of coccidiosis and not tuberculosis.

Hadley (1911) states that Johannes Muller and Retzius, in an account published in 1842, describe "Psorosperms" which were recognized by Remak in 1845 as being the same as the pus globule described by Hake. The formation of sporo- blasts was discovered by Kaufmann in 1847. According to Ziegler (1908) Eimer published observations on the development of coccidia in 1870.

In 1879 Leuckart founded the class Sporozoa and included in it, under the generic name Coccidium, the organism found in the liver of the rabbit by Hake, Muller and
Retzius and Remak. However, the generic name Eimeria previously used in 1875 by A. Schneider holds by Law of priority.

B. Distribution

The first report of bovine coccidiosis was that furnished by Zürn (1878) a Swiss veterinarian, who described a case of acute hemorrhagic dysentery which caused the death of a calf, although Kutyre and Marek (1983) state that Proeger described a disease in calves due to psorospermium in 1877. Zürn did not describe the parasite, but stated that rabbits were not kept with the calves. Rivolta (1878) gave the name Cytospermium Zurnii to the organism which Zürn had observed. Rivolta's original spelling of the specific name should be noted. Rules of nomenclature permit changing the capital "Z" to the lower case "z" but not to drop the final "i" or add an umlaut as has frequently been done in the literature. The generic name Eimeria had been used by A. Schneider in 1875, hence has priority over Rivolta's genus Cytospermium and the correct name is therefore Eimeria Zurnii. He did not describe it further than to state that it seemed identical both with the species found in rats which had been described by Eimer and the psorosperm of rabbits.

The first record of coccidiosis of cattle in America was by Theobald Smith (1893). He states that his observation was made in 1889. He observed white, spherical bodies from
0.3 to 0.4 mm in diameter in the free ends of the villi of the posterior portion of the small intestine and occasionally the cecum in cattle which had died of Texas fever. He subsequently observed the same thing in cattle from a Washington, D.C. abattoir. He recognized them as sporozoae and gives an accurate description of the merozoites and their motility when liberated.

Since Smith's observation, outbreaks of bovine coccidiosis have been reported in North America by Reichel (1910) who saw the disease in cattle, horses and a goat on a farm in Pennsylvania; and by Schultz (1915) who reported the occurrence of coccidiosis in the Pacific Northwest and the Philippine Islands. Reichel's description of the parasite, however, would indicate that his diagnosis was in error and Schultz's paper contains many erroneous statements. Smith and Graybill (1918) studied an outbreak in New Jersey and made what was by far the most accurate and detailed contribution to the literature of the subject yet reported. They believe the disease occurs chiefly in warm weather. The next report of the disease in the United States was by Lentz (1919) who observed several cases in one herd in Pennsylvania. Way and Hagan (1920) saw nine cases in two different herds in New York during the months of November and December. Muldoon (1920) observed an outbreak in one herd of calves in Kansas. Bruce (1921) describes four outbreaks in British Columbia in range
cattle during the winter, of which fifty, or thirty per cent of the animals visibly affected died. Marsh (1923) believes that the disease is quite widespread in Montana, practically all cases occurring in the winter, and estimates that the mortality is about twenty-five per cent of those affected. Davis and Reich (1924) report finding Eimeria oocysts in two cattle slaughtered in California. Gwatkin (1925) diagnosed coccidiosis in the late summer on four different farms near Toronto, Canada. Here the disease ran a mild course, there being no fatalities. Frank (1926) reports several outbreaks of the disease in Kansas and concludes that the degree of transmissibility is very low. His cases occurred during the spring and fall. Barnes and Brueckner (1927) observed several cases in one herd in Pennsylvania. Roderick (1928) states that coccidiosis is widely prevalent in western North Dakota, even estimating that about one-third of the herds in some localities show evidence of the disease each year. He reports that the disease is most prevalent in the winter and that the mortality often ranges from ten to fifty per cent. Becker and Frye (1929) report finding oocysts of the genus Eimeria in three apparently healthy calves in Iowa.

Among others outside of North America who have contributed to our knowledge of bovine coccidiosis should be mentioned Zschokke (1892) of Switzerland who was the first to refer to coccidiosis as a disease of cattle, and Hess (1892)
whose work was equally prominent in Switzerland where the disease was then very common. The work of Guillebeau (1893) is noteworthy in that he mentions two types of coccidia, a larger egg-shaped and a smaller spherical form. Degoix (1904) gives a good description of the symptoms of the disease as it appears in calves in Switzerland while the distinction of being the first to report coccidiosis in cattle from Tunis goes to Ducloux (1905). Zublin (1908) discusses the disease authoritatively based upon an extensive experience with it in Switzerland. Reports of the disease from East and South Africa are made by Montgomery (1910) and Jowett (1911) respectively. Van Hederveen (1925) mentions finding coccidia in apparently healthy animals. Bevan and Kingcome (1925) report an extensive outbreak of coccidiosis in cattle in southern Rhodesia. Nomé (1926) found *Eimeria zurnii* in cattle of Japan. Cooper (1927) reports the disease in cattle of India and Yakimoff (1927) states that coccidiosis in Russia is due to both *Eimeria zurnii* and *Eimeria smithi* and that the mortality may run as high as fifty per cent.

C. Age Incidence

Almost without exception reports indicate that bovine coccidiosis is preeminently a disease of young animals although older animals may occasionally be affected. Law (1911) states that sucking calves are immune and in this Neumann and
MacQueen (1905) agree.

D. Seasonal Prevalence

Reports from Continental Europe indicate that the disease is most frequent there during the warmer parts of the year. Hutyra and Marek (1926) state that it occurs almost exclusively during the warm season. The same has been true in North America in outbreaks occurring in New Jersey, Pennsylvania, Kansas and Ontario, Canada. On the other hand the most extensive losses have been reported as occurring in the winter, notably those in North Dakota, Montana, Kansas, New York and British Columbia. Judging from reports it would seem, however, that the disease may occur at any time of the year and in either wet or semi-arid sections. It is worthy of note that the disease is most prevalent in the winter in the dry sections of the country.

E. Species of the Genus _Eimeria_ Affecting Cattle

The first named species of the genus _Eimeria_ affecting cattle was that described by Zürn (1878) and called _Zurnii_ by Rivolta (1878). This seems to be the one most commonly reported. Smith and Graybill (1918) describe it as having a double contoured wall of uniform thickness, and of circular or ovoid outline. The average dimensions given by them were 18.6μ by 14.8μ. No residual body (Restkörper) was present in either the cyst or the spore after its development. Yakimoff
and Galouzo (1927) agree with this description except that their measurements averaged 17.1µ by 17.1µ.

Concerning the larger forms, Smith and Graybill state: "The oocyst is distinctly ovoid in shape. Sometimes the cyst wall is brownish in color, at other times it is colorless. The wall is thickest at the broad pole and very gradually diminishes in thickness to the small pole. The difference at the two poles is not great yet distinctly noticeable. Some cysts occur, however, in which the wall is uniform in thickness. There is no evidence of a micropyle."
The average measurements given by them were 29.9µ by 19.9µ. There was no residual body (Restkörper) in the oocyst but a large one was present in the spore. Yakimoff and Galouzo (1927) found this species in Russia and named it *Eimeria smithi*. Their description corresponds closely to that given by Smith and Graybill (1918).

Becker and Frye (1929) described a third species - *Eimeria ellipsoidalis* as follows:

"The oocysts of the second species [*Eimeria ellipsoidalis*] encountered only in one calf, were predominantly ellipsoidal. Ovoid or approximately round ones were rarely seen. Although moderately numerous in our smears these oocysts were almost overlooked at first because they were so inconspicuous and colorless. We debated whether they might not be the spores of a fungus. In the fresh oocysts the
protoplasm almost completely filled the cyst. After about four days the protoplasmic mass contracted and became a compact sphere. The fecal matter containing the oocysts was diluted with one per cent potassium dichromate, kept at room temperature and observed at intervals. In about two weeks, four sporoblasts were found in each oocyst. There was no residual body in the cyst. The nuclei of the sporozoites were not visible to us. Measurements gave a length of from 20µ to 26µ and a width of from 13µ to 17µ; average size, 23.4µ by 15.9µ; ratio of average length to average width, 1.47."

Bruce (1921) described what he believed to be another species of Eimeria of cattle and proposed the name Eimeria canadensis. His figures and measurements indicate that he was dealing with more than one species.

F. Physiologic Characteristics

1. Cross Infection Experiments

Attempts to infect one host with the species of Eimeria normally found in another have been numerous and the results conflicting. Zürn (1878) in the first case of coccidiosis reported in cattle stated that rabbits were not kept with calves. Bates (1915) reported a case of coccidiosis in a calf which came in contact with rabbits and which he believed was caused by Eimeria stiedae. Galli-Valerio (1918) attempted to infect cattle with rabbit coccidia but failed. Bruce (1921) concluded that the coccidia isolated
by him from cattle were non-pathogenic for horses, pigs, sheep, rabbits, guinea pigs, white rats and domestic fowls. Uhlhorn (1926) claimed to have succeeded in transmitting coccidia from the rabbit to the chick. He stated that the development of the parasite was retarded in the chick host. Andrews (1927) found that *Isospora felis* and *Isospora rivolte* appeared to be infective to both dogs and cats but with that exception the coccidia of mammals appear to be strictly host-specific as judged by cross infection experiments on cats, dogs, rabbits, skunk, opossum, pig and prairie dogs. Corcuff (1928) believes that the coccidia are host-specific. In a monumental work, Tyzzer (1929) concludes that the coccidia of chickens are highly host-specific. The only cross infection he obtained, after extensive trials, was in turkeys and an occasional slight infection of the chicken with *E. dispersa* of the pheasant. Biester and Murray (1929) fed *Eimeria* recovered from an outbreak of coccidiosis in calves, associated with bloody diarrhea and death, to two pigs weighing about 80 pounds each. Six days after feeding, oocysts appeared in the feces of the pigs in large numbers. Biester and Murray (1930) infected adult chickens with coccidia of swine origin obtained from field sources but were unable to infect swine with coccidia from the chicken or from sheep. They were able to infect swine with oocysts of bovine origin, which upon recovery from the pig, again proved infective for swine and a calf. Oocysts of swine origin did not prove
infective for calves. According to a recent oral communication with Dr. Biester, subsequent trials with oocysts of swine origin having had three passages through pigs kept under experimental conditions, proved non-infective for six to twelve weeks old chicks which had been raised coccidia free.

2. Artificial Excystation

In order to study the viability of coccidial oocysts without passing them through their hosts it is necessary to free the sporozoites from the oocysts. Metzner (1905) was the first to report success in this field. He was able to digest the "Coccidium cuniculi" oocysts with pancreatic and duodenal secretions in a short time with the liberation of sporozoites but had no success with gastric juice. Reich (1913) agrees with Metzner in his work. Lerche (1921) found that the oocysts of the coccidium of the sheep required 17 hours for digestion with duodenal secretions and a much longer time when pancreatin was used. Kolpakoff (1926) furnished evidence that the digestive juices of old rabbits prevents sporogony of Eimeria stiedae but this was not true of young rabbits. Krijgsman (1926) incubated Eimeria stiedae oocysts for 30 minutes at 37° C., then centrifuged and washed the oocyst-containing sediment and added alkalinized trypsin solution. The sporozoites were set free in from 2½ to 6½ hours. Andrews (1930) accomplished excystation by feeding the sporulated oocysts in sweet milk.
to young rats. He stated that he had in this manner digested the oocysts of cats, dogs, guinea pigs, pigs and prairie dogs and that probably any species of coccidia from birds or mammals could be used. He was able to observe the motile sporozoites within and outside the oocysts by microscopic examination of the intestinal content in one hour after feeding. It is worthy of note that the impression gained from this report seems to be somewhat in divergence from that of an earlier report of Andrews (1927), in which he states in his conclusions: "It has been shown that excystation of the oocyst is facilitated by the digestive processes of the natural host, but that in the foreign host oocysts are so resistant to digestive action that the sporozoites are not released during the normal length of time that the organisms pass through the intestine."

3. Effect of Chemicals upon the Oocyst and its Development

Little is known concerning the effect of chemicals upon the species of Eimeria found in cattle. Hutrya and Marek (1926) state that a 5% solution of the cresol compounds will kill bovine coccidia. Perard (1924) in working with Eimeria perforans and Eimeria stiedae stated that they withstand the ordinary disinfectants and acids. Unfortunately, he did not give the strength nor temperature which he used. He did state, however, that they were susceptible to
dessication and were killed at 55° C. in one hour and that 80 per cent were killed in 20 minutes at 55° C. Frank (1926) states that coccidia of cattle are very resistant and are not killed by drying or freezing. Biester and Murray (1929) found that in order to get maximum sporulation it was necessary to aerate cultures in 2 per cent potassium dichromate solution, for at least one minute per day when kept in tubes measuring 205 mm by 25 mm. To each 10 cc of solution 1 gm of bone charcoal was added.

4. Rate of Development

Bruce (1921) states that the time elapsing between infection and the first appearance of blood or oocysts in the feces is about 14 days, and that under the most favorable laboratory conditions, complete development of the oocysts does not occur in less than eight days and may be delayed for weeks or months. Hutyra and Marek (1926) report Deguix as stating that the sporozoites may develop in four or five days or may remain in the feces for two and one-half months without change but retain their capacity for development, that they are very resistant and remain alive after putrefactive processes lasting for three months. They further state that according to various investigators the incubation period is about three weeks. Roderick (1928) succeeded in finding oocysts in the feces of two calves on the 12th and 13th days.
respectively after feeding the infection. Becker and Frye (1929) report that cultures of *Eimeria smithi* oocysts kept in 1% potassium dichromate solution developed four spores each in about two weeks and that *Eimeria ellipsoidalis* was fully sporulated in about 18 days. Both cultures were kept at room temperature.

G. Symptoms

The symptoms of bovine coccidiosis have been well described by workers both in North America and elsewhere. A few references will suffice.

Deguix (1904) stated that the first symptom is the sudden appearance of dysentery with slight rise of temperature, loss of appetite and cessation of rumination. Emaciation is rapid and there is a marked rise of temperature during the latter stages of the disease. The duration of the disease is 5 to 10 days. In favorable cases recovery takes place rapidly. Zublin (1908) stated that coccidiosis is a febrile epizootic of young animals, and that the coccidium is not found in healthy animals. He further stated that recovery is the rule, the mortality being about 5 per cent. The first noticeable sign of the disease is the admixture of blood in the feces. The amount of blood increases and defecation becomes more frequent during the second and third day. The animal may then recover or grow worse. In the latter case the feces become thinner, the amount of blood greater, the
temperature continues to rise, the respirations are increased and the appetite becomes abnormal. From the fifth to the eighth day the feces become thin, watery and fetid and the blood in the feces is gradually replaced by mucus and masses suggesting fibrinous inflammation of the large intestine.

The disease may terminate in death as early as the seventh day after the onset of symptoms.

Schultz (1918) reported that some cases may terminate fatally in two or three days. He further said that the affected animals show a nasal discharge, which may be streaked with blood, and "an inflammation of the eyes." The temperature ranges from 104°F to 107°F and "constipation is the ever present important symptom." Later, diarrhea may be present. Rumination is suspended and drooling of saliva and grinding of the teeth are common. Lentz (1919) sums up the symptoms as follows:

"There was first noticed a serous, fetid, black diarrhea. Fever was rarely in evidence at any time. The diarrhea after a few days changed to mucus, with the passage of blood clots with the mucus and feces from time to time. Straining was very marked. Appetite somewhat impaired but, nevertheless, partook of some food, but finally, in about six to eight days became very dull, refused food, emaciated rapidly, rectum became relaxed, temperature subnormal, pulse hardly perceptible and these symptoms of collapse were soon followed by death."
Way and Hagan (1920) stated that the first symptom noticed is the appearance of a bloody diarrhea which is at first on the outside, but later is mixed with the fecal mass. According to them there is a moderate rise of temperature and pulse rate and the animal appears dull and disinclined to move about.

Muldoon (1920) reported the chief symptoms as being "high temperature, fetid diarrhea, streaked with mucus and blood, lacrimation and muco-purulent nasal discharge."

Bruce (1921) stated that the first symptom may be constipation with mucus and blood in the feces followed by diarrhea containing blood, mucus and shreds of epithelium. He has noticed rapid emaciation, abnormal appetite and grinding of the teeth. There is little or no rise of temperature. Cerebral disturbance was present in a few cases. According to Marsh (1923) the principal symptoms are bloody diarrhea, straining and prolapse of the rectum. There is no appreciable rise of temperature. He also reports occasional evidence of cerebral disturbance.

Van Nederveen (1923) reported finding coccidia in apparently healthy cattle. Becker and Frye (1929) and Smith (1933) reported similar instances. Frank (1926) stated that the chief symptoms are "passage of blood in the feces, with tenesmus, lack of appetite, cessation of rumination and rapid emaciation." Gwatkin (1926) agreed with the observations of Frank and stated that the temperature
Barnes and Brneckner (1927) noticed that in several cases the stricken calves showed a severe bloody diarrhea, at times passing almost pure blood. Straining and attempts to defecate were marked and frequent. The temperature was elevated to 103° F. to 103.5° F. in the worst cases.

H. Pathology

The first pathologic changes observed in bovine coccidiosis of more than superficial examination were those of Smith (1833). He saw small spherical white bodies in the free ends of the villi of the posterior portion of the small intestine, ranging in size from 0.3 mm to 0.4 mm. Occasionally they were seen in the cecum.

Metzner (1903) working with rabbit coccidia was the first to point out that coccidia may invade the subepithelial tissues. Degoix (1904) stated that the lesions of bovine coccidiosis may be found in the whole length of the large intestine and that the etiologic agent is never found in the epithelial cells of the mucous layer of the intestine but in the deeper-lying tissue and in the glands of Lieberkühn.

Jowett (1911) in reporting on one calf says: "The mucous membrane of the small intestine appeared thickened, reddened and swollen and a few superficial erosions and
streak-like hemorrhages were noticed. The reddening of the mucosa was somewhat more evident in the duodenum than elsewhere. The mucous membrane of the large intestine was not uniformly reddened; it was, however, swollen and thickened, and in places was thrown into a number of longitudinal folds. Here, also, streak-like hemorrhages were apparent in places along the folds of the membrane and there was also some patchy reddening of the tissue."

The principal changes found by Smith and Graybill (1918) were in the large intestine although an occasional gland of the small intestine was involved. The lesions appeared as circumscribed foci which tended to coalesce as they increased in size. The glands become denuded of surface epithelium followed by capillary hemorrhage and often necrosis.

Lentz (1919) stated that the lesions of a case autopsied by him were confined to the large intestine. The mucous membrane was reddish-brown in color, soft and spongy and coated with bloody mucus which, when scraped off, revealed large superficial ulcers from the cecum to the anus. Way and Hagan (1920) found, in three cases which they autopsied, that the principal lesions were confined to the large intestine and that the rectum was more involved than the cecum or colon. The small intestine showed a chronic catarrh and in one case the abomasum was congested. Microscopic examination of sections revealed leucocytosis, hemorrhages and sloughing of the glands
over extensive areas.

Bruce (1921) agreed with the above in his observations and stated that the crypts of Lieberkühn were the chief seat of the coccidia but that the intertubular cells may also be invaded. He also stated that the mucous membrane of the abomasum may be eroded in small areas but no coccidia were found there. The blood showed poikilocytosis and polychromasia. A blood count of four animals resulted as follows:

"Monos, 72.12; polys, 21.32; eosins, 6.41; mast, 0.12." He further stated that the parasite appeared to injure only the cell in which it is found.

Yakimoff et al (1926) examined the blood of forty-nine cattle suffering from coccidiosis (Eimeria amnii) and found the leucocytic count to be as follows:

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<th>Young Animals (Up to 1½ years)</th>
<th>Adult Animals</th>
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<td>Lymphocytes</td>
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<td>37.8%</td>
</tr>
<tr>
<td>Neutrophiles</td>
<td>26.3% - 29.0%</td>
<td>38.0%</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>6.2% - 19.0%</td>
<td>20.6%</td>
</tr>
</tbody>
</table>

Their conclusion is that the percentage of eosinophiles is increased by coccidiosis and that this gain corresponds to the increase in age of the animal.

Hutyra and Marek (1926) state that the small intestine of young calves is one of the principal sites of infection but that in older animals the infection is
limited to the large intestine.
III. EXPERIMENTAL

A. Purpose of Study

Most of the information concerning bovine coccidiosis has been gained from field studies. While such information is very valuable, unless it is interpreted in the light of controlled laboratory work, contradictory evidence will appear and confusion will result. Such is the case with bovine coccidiosis. A most cursory review of the literature will reveal many contradictory statements, based largely upon observations of field cases. For the most part the laboratory experiments with this disease have been of a fragmentary nature and have not been done with pure cultures. Moreover, Tyzzer's (1929) work with coccidiosis of chickens has shown that accidental infection with coccidia is very easy, which throws doubt upon much of the laboratory work that has been done. Comparatively little is known of the biology of any of the species of the Eimeria affecting cattle. The same may be said of the pathology of the disease as it appears in cattle. Most of the information upon these subjects has been assumed from what has been learned of the disease in other animals. This is to be expected as cattle are expensive and are not easily handled in the laboratory.
B. Method of Procedure

Throughout the course of this study seven calves, four pigs, three goats, and numerous white rats have been used. All the calves were less than one week of age when they were placed on experiment. The pigs were about six weeks old and weighed between 40 and 50 pounds each. The goats were about four months old.

The room in which the animals were kept during the time they were upon experiment was approximately 20 feet long by 10 feet wide. The walls and ceiling were of brick and tile and the floor was concrete with a drain in the center. The room was supplied with hot and cold running water, high pressure steam, electric lights, and forced draft ventilation. The window and door closed tightly and the latter was kept locked. Six steel cages about 3 by 4 feet in size were arranged along one side of the room. The cages were 4 feet high and the partitions between them were of concrete so that there was no chance for communication between them. The floors of the cages were of wooden slats and were 8 inches above the concrete floor which facilitated cleaning. The room was mouse and fly proof. During critical times of the experiment only those in immediate charge were allowed to enter.

At all times during the experiment the ceiling, walls, floors, cages and feeding troughs were thoroughly
washed every second day with the hose with water at 75° C. When it seemed advisable the washing was done daily and was followed by the use of high pressure steam on the cages, floors and walls.

The pigs were fed a mixture of ground corn and oats which was sterilized with water at 65° C. to 75° C. and all the water given them to drink was taken from the hot water tap. They were given an occasional dose of cod liver oil to prevent dietary deficiency. Whenever the cages and room were cleaned the pigs were scrubbed with warm water and soap.

The calves were fed powdered skim milk which was mixed with water from the hot water tap. Occasionally this was supplemented with fresh milk or raw eggs to prevent digestive disorder and dietary deficiency. The rear parts of the calves were clipped to prevent fecal matter from clinging to them and to facilitate cleaning. At least one uninfected susceptible control animal was kept in the room at all times during the experiment and it is gratifying to state that in not one instance did the control animals become accidentally infected.

During the course of the experiment daily fecal examinations were made of all animals. None of the calves was infected at the time of purchase as was determined by, at least, four negative fecal examinations. The method followed in making fecal examinations was that described by Benbrook (1929). Briefly, this method consists of
mixing a small quantity of fecal matter emulsified in water with a sugar solution composed of 453.5 gms. of cane sugar, 354 cc of distilled water and 3.5 cc of phenol. This is centrifuged for 3 minutes at 600 revolutions per minute. The top film is then lifted off with the end of a glass rod and transferred to a slide for microscopic examination. In using cane sugar instead of a saturated solution of sodium chloride it is necessary to centrifuge the fecal emulsion slightly faster or for a longer period, because of the viscosity of the sugar solution. The specific gravity of the two solutions is approximately the same (1.255 for the sugar and 1.287 for the sodium chloride) but by calculation it is found that the plasmolysis produced by the sodium chloride is three times as great as that produced by the sugar solution. For this reason the sugar solution was used.

The method of isolating single oocysts for observation was in accordance with that described by Barber (1914). In making volumetric measurements of oocysts the method described by Boughton (1923) was used.

Unless stated to the contrary the oocysts were incubated in 2% potassium dichromate solution in a room where the temperature ranged from 27° C. to 30° C. All cultures were aerated at least once daily from 3 to 15 minutes by passing air through a tube into the bottom of the solution from a compressed air apparatus. As the water was lost from
the cultures by evaporation more was added so that the concentration of potassium dichromate was kept at approximately 2%.

The culture of bovine coccidia, hereafter known as culture A, came from a field case of coccidiosis from Grayson County, Virginia. Most of the work of this study was done with this culture or subcultures from it. As will be noted later, it contained three species of Eimeria of bovine origin. Microscopic examination of direct smear of this culture showed an average of 5 Eimeria oocysts per low power field. Culture B was obtained from the cecum and colon of a pig from Iowa suffering from coccidiosis. Culture C was of swine origin and was obtained from the Department of Veterinary Investigation of Iowa State College. Culture D was obtained from a composite fecal sample taken from an Iowa pasture on March 31, 1930. No domestic animals excepting cattle had been in this pasture for several years and none since November 1929. Nonsporulated oocysts of both Eimeria zumii and Eimeria smithi were found but no sporulated oocysts of either were observed. The oocysts were not present in great numbers, but they were sufficiently numerous so there was no difficulty in finding them for observation, when a small amount of fecal emulsion was centrifuged in sugar solution. Culture E was obtained from the rectum of an Iowa cow suffering from an acute attack of bloody diarrhea. This
was a rich culture consisting of about 97% *Eimeria zurii* and 3% *Eimeria smithi*.

C. Results

1. *Eimeria cylindrica* nov. sp.

Early in this study it was noticed that some of the oocysts in culture A did not conform to *Eimeria zurii* or *Eimeria smithi* in size, shape, rate of sporulation, or reaction to freezing temperatures. Measurements were made of 100 consecutive oocysts as they appeared on the slide in a subculture of culture A where they were obtained in pure culture.

<table>
<thead>
<tr>
<th></th>
<th>Major axis</th>
<th>Minor axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>28.8 - 19.4μ</td>
<td>14.9 - 11.9μ</td>
</tr>
<tr>
<td>Mean</td>
<td>23.5 ± 0.24μ</td>
<td>13.3 ± 0.11μ</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.85μ</td>
<td>0.84μ</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>7.86%</td>
<td>6.32%</td>
</tr>
<tr>
<td>Ratio of Major to Minor Axis</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>Content in Cubic Micra</td>
<td>2156</td>
<td></td>
</tr>
</tbody>
</table>

It was not uncommon to find individuals with a major axis twice that of the minor.

The cubical content was calculated by the following formula:
Volume = \( \frac{4}{3} \pi ab^2 \)

where

\( a = \frac{1}{2} \) the major axis

\( b = \frac{1}{2} \) the minor axis

Table I shows a comparison of some of the morphologic and physiologic characteristics of four species of the genus Eimeria affecting cattle. The data for Eimeria zuernii, Eimeria smithi and Eimeria cylindrica were taken from subcultures of culture A.
### TABLE I - A COMPARISON OF SOME MORPHOLOGIC AND PHYSIOLOGIC CHARACTERISTICS OF FOUR SPECIES OF GENUS EIMERIA AFFECTING CATTLE

(Figures 1, 2, 3, 4, 5, 6, 7, 8)

<table>
<thead>
<tr>
<th>Name</th>
<th>Major Axis</th>
<th>Minor Axis</th>
<th>Cubic Content</th>
<th>Residual Body</th>
<th>Time for Sporulation**</th>
<th>Ratio of Major to Minor Axis</th>
<th>Shape</th>
<th>Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eimeria urni</td>
<td>16μ</td>
<td>14μ</td>
<td>1747</td>
<td>None</td>
<td>12-20 days</td>
<td>1.28</td>
<td>Spherical-ellipsoidal</td>
<td>Uniform</td>
</tr>
<tr>
<td>Eimeria smithi</td>
<td>30μ</td>
<td>20μ</td>
<td>6262</td>
<td>In spore</td>
<td>14-21 days</td>
<td>1.50</td>
<td>Egg-shaped</td>
<td>Thin at small pole</td>
</tr>
<tr>
<td>Eimeria ellipsoidalis*</td>
<td>23.4μ</td>
<td>15.9μ</td>
<td>3098</td>
<td>In spore</td>
<td>14-18 days</td>
<td>1.47</td>
<td>Ellipsoidal</td>
<td>Slightly thin micropylar pole</td>
</tr>
<tr>
<td>Eimeria cylindrica</td>
<td>23.5μ</td>
<td>13.3μ</td>
<td>2156</td>
<td>In spore</td>
<td>2-10 days</td>
<td>1.75</td>
<td>Cylindrical</td>
<td>Uniform</td>
</tr>
</tbody>
</table>

*Description from Becker and Frye (1929)

**The time required for complete sporulation is varied by the supply of oxygen and moisture and the temperature.
The shape of *Eimeria cylindrica* is more nearly cylindrical than any other species of *Eimeria* that has been described from cattle. The length of the minor axis is almost the same when taken at any point in the middle one-third of the major axis.

To determine the rate of sporulation, calf No. 8 was given 60 cc of a washed subculture of culture A. This subculture contained *Eimeria zurnii*, *Eimeria smithi* and *Eimeria cylindrica*; the latter two in small numbers. The culture was freshly collected, incubated at 30° C. in 2% potassium dichromate and was fed when 48 hours old. At this time only the *Eimeria cylindrica* appeared, upon microscopic examination, to be fully sporulated. Six days later the feces of the calf were streaked with blood and eleven days after the culture had been given, elimination of *Eimeria cylindrica* in pure culture began and continued until the twentieth day after the culture had been given. The greatest numbers were found in the feces on the seventeenth and eighteenth days.

After the elimination had ceased the calf was given 200 cc of a culture containing the three species under discussion. Eight days later oocysts of *Eimeria zurnii* and *Eimeria smithi* were found in fairly large numbers but no *Eimeria cylindrica* were found. The two former species continued to be found but none of the latter appeared. This would seem to indicate that in the 48 hour culture used only
the *Eimeria cylindrica* were developed enough to be infective and that an immunity to this species, but not to the *Eimeria zurnii* and *Eimeria smithi*, was produced. To show the effect of freezing upon the different species, 30 cc of a non-sporulated mixed culture was refrigerated at -6°C. Seventy-two hours later a portion of this material was removed and placed in the culture room at a temperature of about 30°C. An examination 72 hours later showed that sporulation was proceeding at a normal rate in all three species. After 80 days of refrigeration another sample was placed in the culture room for sporulation. An examination 72 hours later showed, that of the individuals examined, about 90% of the *Eimeria zurnii* and *Eimeria smithi* were developing at a normal rate while all but 10% of the *Eimeria cylindrica* were undeveloped. Culture A514-81 was collected and incubated for seven days at 25°C-30°C, then washed and stored at 6°C. Several examinations showed only *Eimeria cylindrica* present. Three hundred cubic centimeters of this culture was given to calf No. 14. Eighteen days later it began the elimination of *Eimeria cylindrica* oocysts in the feces unmixed with any other species.

For this species the name *Eimeria cylindrica* is suggested.
2. Cross Infection Experiments

a. Swine

Four apparently healthy pigs, numbered from one to four inclusive, were placed in the cages heretofore described. Although these pigs appeared perfectly normal in every way, fecal examination revealed that they were suffering from a mild infection of coccidia, *Ascaris lumbricoides* and *Trichuris suis*. Daily fecal examinations of each animal showed that pig No. 1 eliminated a few Eimeria oocysts until the seventeenth day after being placed in the cages. Pig No. 2 stopped elimination on the eighth day while numbers 3 and 4 became free after the seventh day.

On the thirty-first day (counting from the time the pigs were placed in the cages) numbers 3 and 4 were each given 20 cc of culture A, which was washed free from the preservative - potassium dichromate. This was accomplished by centrifuging at 600 revolutions per minute for two minutes, then pouring off the supernatant fluid. The oocyst-containing sediment was then thoroughly mixed with distilled water and centrifuged again. This process was repeated twice, when it was found that not enough potassium dichromate remained to be toxic to the experimental animals. Such treatment, as will be shown later, did not prove injurious to the oocysts. A direct smear of this culture showed an average of five Eimeria oocysts per low power field. About
90% of these were identified as *Eimeria zumii*, 7% as *Eimeria cylindrica* and 3% as *Eimeria smithi*. Pigs numbers 1 and 2 were untreated to serve as controls.

Daily fecal examinations of all four pigs remained constantly negative for *Eimeria* oocysts.

On the thirty-ninth day pigs numbers 1 and 2 were each given 100 cc of culture A. That given to No. 1 had the potassium dichromate removed by dialysis while that given to No. 2 was washed by centrifuging. Both lots were examined microscopically after the removal of the potassium dichromate to make certain that the *Eimeria* oocysts were present. Daily fecal examinations for *Eimeria* oocysts continued to be negative. On the sixty-third day (14 days after having received culture A) pig No. 1 was given an amount of culture C equal in oocyst content to 40 cc of culture A, from which the potassium dichromate had been removed by dialysis. Culture C was coccidia of swine origin obtained from the Department of Veterinary Investigation, Iowa State College. On the sixty-fifth day pig No. 2 was given an amount of culture B (coccidia of swine origin obtained from a field case) equal in oocyst content to 12.5 cc of culture A. The potassium dichromate was removed from this culture by dialysis. At the same time pig No. 3 (34 days after having received the bovine coccidia--culture A) was given the same amount as pig No. 2 of culture B which in addition to having
the potassium dichromate removed by dialysis was centrifuged and washed as heretofore described. Pig No. 4 which had received coccidia of bovine origin (culture A) 34 days previously remained untreated as a control.

On the seventieth day, (seven days after receiving culture C of swine origin) No. 1 began to eliminate Eimeria oocysts. On the seventy-fifth day, (ten days after receiving culture B of swine origin) numbers 2 and 3 began to eliminate Eimeria oocysts. This was continued by pigs numbers 1, 2, and 3 until disposed of on the seventy-seventh day. At this time pig No. 4 remained negative to fecal examinations for Eimeria oocysts.

b. Goats

Culture A5L2-16, a subculture of culture A, of bovine origin containing *Eimeria zumii*, *Eimeria cylindrica* and *Eimeria smithi* was given to three goats about four months old. A dose of 100 cc of the culture was given to each goat on three consecutive days. The results are not entirely satisfactory as no goats could be found that were not infected with coccidia. However, in view of the heavy dosage and the fact that no Eimeria recognizable as bovine species were eliminated for a period of 21 days it would seem that they were not susceptible to the culture used.
3. Effects of Temperature

a. Maximum

Nonsporulated subcultures of culture A, 24 hours old were heated in a water bath for 10 minutes at the temperatures indicated in Table II. After heating, 2% potassium dichromate solution was added and the cultures were placed in the incubation room for sporulation. The results of examination for development is indicated in Table II. This was repeated three times with different subcultures of culture A. No change could be noted in the microscopic appearance of the oocysts even when heated to 75° C. for 20 minutes (Figure 9). The percentages indicate the number showing sporulation.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>1st Trial</th>
<th>2nd Trial</th>
<th>3rd Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>75° C.</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>55° C.</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>60° C.</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>55° C.</td>
<td>0%</td>
<td>0%</td>
<td>10%*</td>
</tr>
<tr>
<td>50° C.</td>
<td>50%</td>
<td>47%</td>
<td>48%</td>
</tr>
<tr>
<td>45° C.</td>
<td>80%</td>
<td>85%</td>
<td>85%</td>
</tr>
</tbody>
</table>

*Later examination showed that development started but did not continue.*
Nonsporulated culture A55 was divided, one part being incubated at 37° C. and the other at 30° C. No difference could be noted in the rate of development.

b. Minimum

On January 16, 1930, a fully sporulated portion of culture A was placed outdoors where it would be exposed to outdoor temperature. A portion of this culture was examined February 27, 1930, and by microscopic examination appeared to be unchanged. On April 10, 1930, the culture still appeared to be normal excepting the oocyst wall which seemed to be somewhat thickened and spongy (Figure 10). The remaining portion (10 cc) was given to calf No. 3 after the potassium dichromate had been removed by centrifuging and washing. No infection resulted. Further evidence of the destructive effect of low temperatures is furnished by the observations made on culture D. It will be recalled that this culture was obtained on March 31, 1930 from a pasture upon which there had been no domestic animals since the preceding November, 1929 and then only cattle. Only non-sporulated oocysts could be found but these appeared, microscopically, to be normal. This culture was incubated at 30° C. in 2% potassium dichromate solution and aerated daily. No development took place and the culture was discarded in ten days, as dead.
The effect of \(-6^\circ\) C. temperature upon non-sporulated cultures of Eimeria oocysts is shown in Table III. A subculture of culture A was placed in the refrigerator and portions taken out and placed in the incubation room at \(30^\circ\) C. for development. Observations of the development were made after 72 hours of incubation. The culture was in 2\% potassium dichromate. A development of 95\% may be regarded as perfect as some oocysts are usually sterile.

**TABLE III - EFFECT OF \(-6^\circ\) C. TEMPERATURE UPON THE VIABILITY OF EIMERIA OOCYSTS WITH SUBSEQUENT INCUBATION AT 25\(^\circ\)-30\(^\circ\) C.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Number of days exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td><em>E. nurnii</em></td>
<td>85%</td>
</tr>
<tr>
<td><em>E. smithi</em></td>
<td>95%</td>
</tr>
<tr>
<td><em>E. cylindrica</em></td>
<td>95%</td>
</tr>
</tbody>
</table>

At \(-6^\circ\) C. none of the above species showed any development, while development at \(6^\circ\) C. proceeds very slowly.

4. Effect of Centrifuging

A mixed nonsporulated culture No. A55 was divided and one part centrifuged for ten minutes at 1000 revolutions per minute. Both portions were placed in the incubator room for development. No difference either in the rate or
percentage of sporulation could be detected; 90% of all three species developed in the normal time.

5. Effects of Some Disinfectants

Freshly collected oocysts of *Eimeria zurnii*, *Eimeria smithi* and *Eimeria cylindrica* were treated with disinfectants as indicated in Table IV. In all cases the fecal material was passed through a 40 mesh screen to remove the larger particles, so that all the oocysts would be exposed to the action of the disinfectant. In case the cultures were not incubated in the disinfectant, it was removed by centrifuging and washing and 2% potassium dichromate added to prevent putrefaction. Table IV represents an average of three trials with three different subcultures and shows the percentage of viable oocysts on examination 72 hours after treatment (Figures 3 and 7). The temperature at which the experiment was conducted ranged from 25° C. to 30° C.

<table>
<thead>
<tr>
<th>TIME OF EXPOSURE</th>
<th>72 HOURS</th>
<th>1 HOUR</th>
<th>10 MINUTES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHENOL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent sporulated</td>
<td>95:0:0</td>
<td>95:88:0</td>
<td>Not</td>
</tr>
<tr>
<td><strong>MERCURIC CHLORIDE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent sporulated</td>
<td>0:0:0</td>
<td>0:0:0</td>
<td>70:90</td>
</tr>
</tbody>
</table>

No development took place when the cultures were incubated in 4% formaldehyde or 2% Liquor Cresolis Compositus, but 50% of the oocysts developed in 2% copper sulphate.
Ninety-five per cent sporulation is considered perfect as at least 5% of the oocysts are sterile.

6. Effect of Sunlight and Drying

Twenty-four hour nonsporulated cultures of coccidia of bovine origin (subcultures of culture A) were poured in four open Petri dishes and dried. In two of the dishes no preservative was used. In the other two 2% potassium dichromate was added. One dish without and one with potassium dichromate were placed in the incubation room. The other two dishes were exposed to the direct rays of the sun for a total of about 24 hours. Three days later water was added to the four cultures. No development took place in either of the cultures exposed to the sun while the viability of the oocysts dried but not exposed to the sun was impaired in three days and was reduced to 50% after 15 days drying. This test was repeated with similar results but in both instances the humidity in the incubation room ranged between 80 and 90 for most of the time, hence the oocysts could not be considered as thoroughly dried.

7. Effect of Putrefaction

In working with coccidia it has frequently been observed that putrefaction, especially when blood is present in the feces, kills the organism in a short time. This was
shown in one case when calf No. 5 was having a considerable amount of intestinal hemorrhage produced by coccidia. Freshly collected fecal matter containing the oocysts was divided in two bottles and placed in the refrigerator at -6° C., after 2% potassium dichromate solution had been added to one of the bottles. In a few days, in spite of the fact that the cultures were frozen, the one without the preservative had a noticeable odor of putrefying blood. At various intervals some of both cultures was taken out and potassium dichromate added to the one without it and both placed in the incubation room. After 35 days only about 50% of the *Eimeria zurnii* and *Eimeria smithi* were viable in the culture without the preservative and 85 to 90% in the culture containing the preservative. It is to be borne in mind that not a great amount of putrefaction can take place at -6° C.

8. Artificial Digestion

Fifteen cubic centimeters of fully sporulated culture A were washed free of potassium dichromate by centrifuging in three changes of distilled water and allowed to stand in distilled water for 14 hours to remove any trace of the potassium dichromate. This was again centrifuged and the oocysts were separated from the sediment by centrifuging in sugar solution. The upper portion was removed, mixed with distilled water and again centrifuged. This left the *Eimeria* oocysts in the bottom of the centrifuge tube almost entirely
free of fecal matter and other debris.

Two cubic centimeters of a solution containing pepsin (Merck) 0.3% and hydrochloric acid (C.P.) 0.2% were then added to the washed and concentrated oocysts. This was incubated for two and one-half hours at 37°C. Microscopic examination after one hour incubation showed that the cyst wall was slightly thickened and appeared to be spongy. This was further shown when the cover-glass was pressed against the slide since all of the sporocysts could be seen in one plane. By exerting considerable pressure upon the cover-glass about 40% of the oocyst walls were ruptured and the sporocysts were liberated, but no sporozoites were observed free from the sporocysts. The pepsin and hydrochloric acid solutions were then removed by washing and 2 cc of a solution of trypsin 0.3% (Merck) and sodium carbonate (C.P.) 0.25% was added. This was incubated at 37°C. An examination three hours later showed no further digestion, i.e., by pressing firmly upon the cover-glass 40-50% of the sporocysts could be removed from the oocyst by rupture of its wall but no sporozoites could be liberated from the sporocysts. Daily examinations for fifteen days showed no further digestion (Figure 11).

This experiment was repeated three times; the results being the same in each case. In another instance the trypsin 0.3% and sodium carbonate 0.25%, without the
pepsin and hydrochloric acid, was tried but other than softening the oocyst wall, no results were obtained (Figure 12). In still other cases pancreatin (Merck) and fresh bile were used instead of trypsin and sodium carbonate but the results were the same. No difficulty was experienced in freeing a high percentage of the sporozoites of Eimeria from the chicken by these methods (Figures 13 and 14).

Washed and concentrated, fully-sporulated oocysts from subcultures of culture A were mixed with milk and fed to young white rats that had been starved for 24 hours. The rats were killed in from one to two hours after feeding and the contents of the small intestine examined for Eimeria oocysts. No difficulty was experienced in locating the oocysts but in not one instance were sporozoites seen. When this was tried with Eimeria of the chicken, sporozoites were found.

Further evidence bearing upon digestion of Eimeria of bovine origin was shown in repeated experiments with a young white rat in which a fistula of the ileum was established. This was done by disconnecting the ileum from the cecum and attaching it to the floor of the abdominal wall allowing the content of the intestine to flow out through the opening. Cultures of coccidia of bovine origin were fed to the rat but no sporozoites were liberated. Coccidia of chicken origin were digested and the sporozoites seen.
9. Pathogenesis Following Artificial Infection of Calves

Calf No. 5, when one week old, was given 75 cc of culture A. The elimination of oocysts resulting from this infection may be divided into two periods; the first, beginning on the seventh day and ending on the eighteenth, and the second, starting on the twenty-sixth day after the original culture was given and ending on the forty-ninth. Thus, there was an interval of eight days between the two periods when no oocysts were found.

On the seventh and eighth days all the oocysts examined were clearly Eimeria zurnii. The first oocysts of Eimeria smithi were found on the ninth day and continued until the close of the first period, the eighteenth day. During this period the Eimeria smithi formed about 20% of the total oocysts. From the twelfth to the fifteenth days inclusive, Eimeria cylindrica were found ranging from 1% to 20% of the total.

The second period, commencing with the twenty-sixth day, showed the heaviest infection as indicated by the number of oocysts passed. During the twenty-sixth, twenty-seventh, and twenty-eighth days the oocysts, which were about 70% Eimeria zurnii, were too numerous to count. Hemorrhage from the intestine was marked but not profuse. On the thirtieth to the thirty-third days the number of
Eimeria cylindrica was greatly increased; a pure culture being recovered from one fecal passage on the thirtieth day. The elimination of Eimeria zurnii and Eimeria cylindrica ceased on the thirty-fifth day after the original infection and from then on to the forty-ninth day pure cultures of Eimeria smithi were eliminated.

On the fiftieth day 150 cc of a mixed subculture of culture A was given but no infection resulted until three weeks later when a mild chronic infection with Eimeria zurnii and Eimeria smithi resulted. No Eimeria cylindrica oocysts were observed in the slides examined, although it is possible that some may have been present in passages of feces which were not examined.

Calf No. 7 was given 60 cc of a 24 hour old, pure culture of Eimeria cylindrica. On the fourth and fifth days, the feces were streaked with blood and on the eighth day a few Eimeria cylindrica oocysts appeared in the feces. The eighteenth day was marked by the animal suffering from a fibrino-hemorrhagic enteritis which lasted for four or five days but no oocysts could be found in the feces. On the nineteenth day 150 cc of a mixed subculture of culture A was given by means of the stomach tube. No oocysts appeared in the feces until three weeks later when a few Eimeria zurnii and Eimeria smithi were observed. On the twenty-third day after the second infective dose Eimeria smithi were
eliminated in large numbers and the calf was killed for autopsy. Oocysts of *Eimeria smithi* were found in approximately equal numbers in the contents of the ileum, cecum and colon.

Sixty cubic centimeters of a 48 hour subculture of culture A, containing 80% *Eimeria smithi*, 10% *Eimeria zurnii* and 10% *Eimeria cylindrica* was given to calf No. 8. From the fifth to the seventeenth days, the feces were markedly streaked with blood and *Eimeria cylindrica* oocysts in pure culture were found from the eleventh to the eighteenth days, appearing in greatest numbers on the seventeenth day. As no oocysts appeared on the nineteenth day, the calf was given 200 cc of a mixed subculture of culture A. Nine days later blood appeared in the feces and continued until the animal was killed for autopsy on the twelfth day after receiving the mixed culture. Two days previous to the autopsy *Eimeria zurnii* oocysts were found in the feces in pure culture in great numbers and were present in the content of the cecum, colon and rectum at the time of autopsy, though not in great numbers.

Calf No. 9 received 60 cc of an almost pure culture of *Eimeria zurnii* although *Eimeria smithi* and *Eimeria cylindrica* were present in small numbers. Seven days later a few *Eimeria zurnii* oocysts appeared in the feces and continued in small numbers until the seventeenth day when they suddenly became too numerous to count (about 200 per low power
field on smears made from sugar-centrifuged suspensions of feces). About 90% were Eimeria zurnii and 10% of about equal numbers Eimeria smithi and Eimeria cylindrica. On the eighteenth day the number of oocysts was about the same as on the previous day but the number of Eimeria cylindrica had increased to about 45% and the number of Eimeria zurnii had fallen to about 45%. On the twentieth day 200 cc of a mixed culture was given the calf but the number of oocysts gradually decreased until on the forty-first day only a few oocysts were being eliminated. There was no response to the second, 200 cc dose.

Calf No. 10 was given 60 cc of a lean culture in which at least 95% of the coccidia were Eimeria smithi. A few Eimeria cylindrica and Eimeria zurnii were present but their number did not exceed 5% of the total. This culture was incubated for eleven days at 25° C. to 30° C. and aerated daily. It was then stored for 5 days without aeration at 6° C. The culture was thus 14 days old when used but had been at a temperature high enough for development to take place for only eleven days. On the sixteenth day after receiving the infective dose the elimination of Eimeria cylindrica commenced and continued in small numbers until the twenty-first day, at which time there was a moderately heavy elimination of about equal numbers of Eimeria zurnii and Eimeria cylindrica. Previous
to this, on the twentieth day, the animal received 400 cc of a nearly pure culture of *Eimeria zurnii*. Eight days later the elimination of *Eimeria zurnii* oocysts began in fairly large numbers and continued in about the same numbers until the eighteenth day when a few *Eimeria smithi* were eliminated.

Calf No. 14 was given 500 cc of a pure culture of *Eimeria cylindrica*. Eighteen days later it commenced to eliminate this species in pure form.

10. Symptoms

Based upon observations of scores of field cases and upon six calves artificially infected it may be safely stated that the predominant symptoms of bovine coccidiosis are catarrhal, hemorrhagic diarrhea, general anemia, and emaciation. The temperature remains normal or becomes subnormal. A rise of temperature is indicative of secondary bacterial infection. The animal becomes dull, listless and weak. The appetite remains fair although there is evidence of digestive disorder as indicated by drooling of saliva and grinding of the teeth. Respiration remains normal unless the animal develops pneumonia which often occurs if ample protection is not afforded. The pulse becomes rapid and thread-like, the eyes are sunken and the hair coat becomes dull.
The pathologic changes in bovine coccidiosis are primarily confined to the intestine, principally the cecum, colon and rectum. The most noticeable gross lesions are loss of surface epithelium, hemorrhages and mucosal thickening. The hemorrhages vary from petechiae in mild infections to diffuse hemorrhages in severe acute infections. In the latter case the intestinal mucosa is reddish-brown. The affected areas of mucosa are thickened and form irregular ridges or corrugations, due to infiltration with leucocytes and lymph. The crests of the ridges are hyperemic and hemorrhagic. So far as observed the parasites invade chiefly the epithelium of the deeper portions of the intestinal glands where they may be found in all stages of development (Figures 16, 17, 18, 19 and 20). The rectum usually shows the most extensive involvement although the colon and cecum show more or less change similar to that found in the rectum. Catarrhal enteritis usually occurs in both the large and small intestine and in some animals catarrhal abomasitis is present. The mesenteric lymph nodes are enlarged and juicy and are hyperemic when there is secondary bacterial infection.
IV. DISCUSSION

In the study of any infectious disease it is of prime importance to know as much as possible about the morphologic and physiologic characteristics of the etiologic agent. It is equally important to be able to recognize and separate the different species and in most cases it is necessary to use pure cultures in order to avoid confusing results. For these reasons a considerable amount of time has been spent in an attempt to gain information of a fundamental nature concerning the etiologic agents of bovine coccidiosis.

One of the results of this study has been the discovery of a species, viz. Eimeria cylindrica, the oocysts of which may reach the infective stage after 48 hours development, under favorable conditions. Failure to recognize this would lead to confusion in experimental work with bovine coccidia, and will necessitate a revision of sanitary control recommendations. It also explains some of the failures of present day measures of control.

In describing an organism like an Eimeria oocyst it should be borne in mind that it possesses three dimensions, not two, as most of the descriptions in the literature might lead one to believe. Because the Eimeria oocyst does possess three dimensions the terms major and minor axes have been used and the cubic content has been calculated. This
concept of the oocyst is more accurate than to consider it as flat, and having two dimensions.

It is highly important to know whether swine will contract the infection from cattle suffering from coccidiosis. It seems to be clearly shown that the four pigs used in this study were not susceptible to the species of Eimeria in culture A. It is to be noted that, after failure to infect the pigs with this culture, the pigs were proven to be susceptible to Eimeria of swine origin and the cattle culture was proven to be viable by feeding to calf No. 5. It was thought at first that either the culture was dead or that the pigs were not susceptible to Eimeria infection. Subsequent events, however, proved neither to be the case. It is to be borne in mind that Biester and Murray (1929 and 1930) used bovine coccidia of Iowa origin and if any species or strains of bovine coccidia will infect swine it would be expected that they would be found in the Central West where cattle and hogs live in great numbers in close proximity.

It seems quite evident that the nonsporulated oocysts of the three species of bovine coccidia studied are not as resistant to extremes of temperature, disinfectants, sunlight, and putrefaction as is generally believed. As a matter of fact, the thermal death point (55° C.) is very low as compared with most other cystic forms of microscopic life. The minimum thermal death point is also
comparatively high. These facts, together with what has been shown relative to the destructive action of putrefaction and the rays of the sun, immediately raise the question as to how the disease can exist in cold climates. The answer is probably that there are chronic carriers of the infection. It is undoubtedly true that the infection is far more common in chronic carriers than is generally believed.

The action of mercuric chloride is so effective in destroying nonsporulated oocysts that there are many cases where it can be made of practical value in destroying the infection. Sunlight and hot water or steam can also be used to advantage in many cases.

It is not surprising that it was found impossible to digest bovine coccidia oocysts in vitro, or in live rats. Disregarding the fact that Eimeria oocysts may require the digestive juices of their normal hosts for their digestion, it is to be expected that in a ruminant the digestion of the oocyst wall is greatly augmented by the maceration which it undergoes during its journey through the rumen, which may last for several hours or for as many days. This may also explain why many observers have stated that suckling calves are immune to the infection. It is, of course, known that the rumen does not function until the calf starts eating solid food. It is possible that this explains why the calves used in this experiment did not become more heavily infected.
Many undigested, fully sporulated oocysts could be found in the feces of the experimental calves in from 24 to 48 hours after they received the infecting dose.

A study of the results of artificial infection with bovine coccidia shows that about the seventh day oocysts generally appear in the feces. This phase of the disease is often preceded or accompanied by intestinal hemorrhage. This is followed by a lag in the number of oocysts appearing in the feces until about the eighteenth to twentieth days when again there is a marked rise. During the peak of the second period the oocysts are eliminated in numbers twice or three times as great as during the first period. In some cases few or no oocysts are eliminated during the first period but the infection is marked by hemorrhage. A logical explanation of this would seem to be that about 5 days after infection the first crop of merozoites matures. Some of these may undergo sexual development and form oocysts while others undergo asexual development, appearing again as merozoites about the fourteenth day. At this time many undergo sexual development while a few appear to again undergo asexual development. This raises the question as to whether the resistance or immunity of the host has any influence in determining whether development of the merozoites will be sexual or asexual.
V. CONCLUSIONS

1. There are many contradictory statements in the literature concerning bovine coccidiosis.

2. *Eimeria cyindrica* nov. sp. is described. The most outstanding physiologic characteristic of this species is the short time required by the oocyst for sporulation.

3. Cross-infection experiments with swine and goats indicate that the species of *Eimeria* used in this study are highly host specific.

4. The maximum thermal death point of nonsporulated oocysts of *Eimeria zurnii*, *Eimeria smithi* and *Eimeria cylindrica* is about 55° C. with an exposure of 10 minutes.

5. Winter temperatures as encountered in Iowa destroyed both sporulated and nonsporulated oocysts of three species of bovine coccidia.

6. A temperature of -6° C., after 35 days exposure, slightly reduced the number of nonsporulated oocysts of *Eimeria zurnii* and *Eimeria smithi* that developed at 25°-30° C. Such treatment made a much greater difference in the number of *Eimeria cylindrica* oocysts that developed.

7. Centrifuging nonsporulated oocysts for 1000 R.P.M. for 10 minutes did not appear to reduce the number or retard the rate of sporulation of the three species of bovine coccidia that have been studied.
8. Nonsporulated bovine coccidial oocysts are readily destroyed by mercuric chloride, phenol, liquor cresolis compound and formaldehyde, but were not destroyed when incubated in 2% potassium dichromate or 2% copper sulphate.

9. Drying in the direct rays of the sun for 24 hours killed Eimeria oocysts of bovine origin. Drying, out of the sun, was less effective.

10. There is indication that putrefaction readily destroys nonsporulated oocysts of bovine origin in a short time.

11. Fully sporulated coccidia of bovine origin proved difficult or impossible to digest by ordinary means in vitro or with young rats. Young calves fed on powdered skim milk were not immune to bovine coccidia, although they were somewhat resistant, probably because the oocyst did not remain in the body long enough for digestion.

12. When fully sporulated oocysts were given to susceptible young calves, nonsporulated oocysts appeared in the feces in about seven days. About five days later there was a decline in the number eliminated, which was followed by a secondary rise which reached its peak about eighteen days after the original infective dose was given. During the secondary rise, the number eliminated was twice or three times as great as during the primary period.
13. An acute attack of bovine coccidiosis may end in the animal becoming a chronic carrier for an indefinite length of time.

14. Possibly resistance or immunity is a greater limiting factor to the disease than the fact that there may be a limited number of asexual generations of the organism.

15. The strict sanitary precautions practiced in this study prevented the spread of coccidiosis to susceptible calves.
VI. DESCRIPTION OF PLATES

Plate I.
The first three figures of this plate show three stages in the development of *Eimeria cylindrica*. Figure 3 shows the organism as it is often seen after 48 hours development under favorable conditions. Note the characteristic shape of the oocyst. x2200.

Figures 4 and 5 are photomicrographs of *Eimeria cylindrica* after 48 hours incubation at 30° C. in 2% potassium dichromate. The minor axis has been considerably lengthened by the cover-glass being pressed against the slide. x2180.

Plate II.
Figure 6, a photomicrograph of two oocysts of *Eimeria smithi* and one of *Eimeria zurii*. These are from a culture incubated for 7 days in mercuric chloride 1–1000. The mercuric chloride produced some coagulation of the protoplasm but the size and shape of the oocyst has not been changed. x2180.

Figure 7, a photomicrograph of two oocysts of *Eimeria zurii*. These oocysts have been treated the same as those in figure 6. x2180.

Plate III.
Figure 9, photomicrograph of *Eimeria ellipsoidalis* preserved in formaldehyde. x2180.

Figure 9. Fully sporulated *Eimeria zurii* oocyst from culture A which was heated to 75° C. for 20 minutes. x650.
Plate IV.

Figure 10. Two oocysts of *Eimeria zurnii* from culture A when exposed to outdoor temperature from January 16, 1930 to February 27, 1930. x700.

Figure 11. Two fully sporulated *Eimeria zurnii* oocysts incubated for 2½ hours at 37° C. in a solution of pepsin 0.3% and hydrochloric acid 0.2%, followed by 24 hours incubation at 37° C. in a solution of trypsin 0.3% and sodium carbonate 0.25%. x1080.

Plate V.

Figure 12. Two *Eimeria zurnii* oocysts incubated at 37° C. for 2½ hours in a solution of trypsin 0.3% and sodium carbonate 0.25%. x650.

Figure 13. *Eimeria* oocyst from the chicken. The oocyst wall has ruptured and the sporocysts are escaping. Several sporocysts are seen. Excystation was accomplished by pepsin 0.3% and hydrochloric acid 0.2% for 15 minutes followed by bile and pancreatin 0.5% and sodium carbonate for 1 hour at 37° C.

Plate VI.

Figure 14. The same as figure 13. Two sporocysts and one sporozoite are seen. x840.

Figure 15. Mixed culture of bovine coccidia. Note the malformed oocyst. x650.
Plate VII.
Figure 16. Macrogametocyte. Ileo-cecal valve. x125.
Figure 17. Same as figure 16. x500.

Plate VIII.
Figure 18. Macrogametocyte. Ileo-cecal valve. x1000.

Plate IX.
Figure 19. Macrogametocyte. Colon. x125.
Figure 20. Same as figure 19. x1000.
PLATE III

Figure 8

Figure 9
PLATE VI

Figure 14

Figure 15
VII. LITERATURE CITED


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