A STUDY OF BOVINE COCCIDIOSIS

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I. INTRODUCTION

Very little exact knowledge existed concerning coccidial infections before the appearance of Tyzzer's report on the coccidia of gallinaceous birds. Reports in literature on this subject written during the past ten years show that the occurrence of coccidiosis is widespread in live stock and poultry, both in this country and abroad. With increasing knowledge of coccidiosis, live stock owners are beginning to recognize the disease and realize the seriousness of the yearly economic losses that result from it. It is surprising that the need for the investigation of this condition has escaped the notice of scientific workers for so long a time. Bovine coccidiosis has received scant attention because as a rule the percentage of fatalities resulting from it is not high. The unthriftyness and lack of growth in the parasitized animals usually pass unnoticed.

Four species of the genus Eimeria of cattle have thus far been described by investigators. Wilson's extensive work with bovine coccidiosis has established many of the uncertain physiologic characteristics of the species parasitic to cattle. The recent work of Fish shows the possibility of error in attempting to recognize

the different species by their morphologic characteristics alone. There is more need of careful study of the physiologic characteristics of each species and the associated pathologic changes produced.

II. REVIEW OF LITERATURE

A. Historical

Nodular formation in the bile duct of the rabbit as a result of coccidial infection was noted by many early investigators. Carswell (1838) published a colored drawing of this condition, which he considered to be a tubercule. Hake (1839) was the first observer to definitely describe the coccidial oöcyst, which he found in the liver and duodenum of a rabbit. Hake is usually given credit for the discovery of the coccidia, but Dobell (1902) has pointed out that Leenwenhoek, in an unpublished letter dated 1674, gave some indications that he had seen the oöcysts in the bile of rabbits. The presence of oöcysts in the intestinal mucosa was first noted by Remak in 1845. Rayer (1846) and Dujardin (1846) regarded them as immature eggs of a trematode. Kauffmann (1847) first noted that if the oöcysts were kept in water the contents separated into four separate bodies. In 1865 Stieda observed the development of the oöcysts and concluded that they were early developmental stages of an unknown parasitic organism. In the same year Lindemann definitely regarded the organism as a parasite. Leuckart (1879) founded the class Sporozoa, and included this organism under the

generic name coccidium, but this is a synonym of Eimeria, which was the generic name used by A. Schneider in 1875.

Balbiani (1884) gave an accurate description of the development of the oöcyst. He showed that the contents segmented into four sporocysts which eventually produced a residual body and two sporozoites. The fact that there occurred an endogenous multiplication followed by the production of oöcysts within the epithelial cells of the rabbit was first pointed out by R. Pfeiffer (1893). He believed, as Eimer, that the oöcysts were the infective forms, while the stages within the epithelial cells brought about a multiplication of the parasite within the host. These views were strongly contested by Schneider (1892), Labbé (1896), and others who believed that the two stages represented two distinct parasites. The facts of the life history of coccidia were definitely established by the researches of Schaudin (1900) on Eimeria schubergi. Cattle coccidia were first seen by Zürn, a Swiss veterinarian, in 1878. They were given the name Cytospermium zurnii by Rivolta (1878). The correct name for the parasite becomes Eimeria zurnii Rivolta (1878). Eimeria was the generic name used by A. Schneider in 1875.

B. Distribution

An acute enteritis or dysentery of cattle associated with coccidia was first noticed by Zürn (1878). According to Hutyra and Marek (21), Proeger had in 1877 described a disease in calves due to psorospermium. Wenyon (37) states that Zschokke (1892), Hesse (1892), and Guillebeau (1893) were the first to describe a definite coccidiosis of cattle as a distinct disease in Switzerland. Theobald Smith (31) published in 1889 the first account of bovine coccidiosis in this country.

Jowett (23), gives measurements of cattle coccidia observed by him. These were consistently smaller than the ones noted by Guillebeau. The variations in the size of the oöcysts were noted by Smith and Graybill (32) in an outbreak of coccidiosis in New Jersey. The smaller type of oöcysts were consistently found to be more numerous than the larger type. They believed that the calves contracted the infection soon after birth. The first symptoms of the actual dysentery appeared from three to six weeks later, and the disease ran a course of six to eight weeks. An occurrence of coccidiosis in Pennsylvania was reported by Lentz (25).

Cases of coccidiosis have been reported from New York, Kansas, Montana, Iowa, and British Columbia. The variation in size of the

oöcysts found in cattle in California was reported on by Davis and Reich (12). Wilson (38) reports that coccidiosis is so prevalent in some sections of Virginia and adjoining states that it is almost a limiting factor in the beef cattle industry.

Coccidiosis has been reported in various countries in Europe, in Asia, South America, and Africa. The disease is probably universal in distribution.

C. Age Incident

Law (24) has reported that suckling calves are immune. Wilson (38) believes that slight infections in young animals result from the fact that the rumen is not functioning and that a majority of the oöcysts pass through the animal without being digested. Reports in the literature are agreed that coccidiosis is primarily a disease of young animals, although older animals may be affected.

D. Seasonal Prevalence

The literature gives proof that coccidiosis may occur at any season of the year in either wet or dry climates. Smith and Graybill (32) believe that the disease is more prevalent during the summer months. The outbreaks of the disease in Europe occur almost exclusively during the spring and summer, according to Hutyra and Marek (21). Way and Hagan (36) saw two outbreaks in New York in the winter. Bruce (11) reported on a number of cases observed in British Columbia during the winter with a high percentage of mortalities. According to Marsh (26) the disease occurs in Montana entirely during the winter. This seems to be true of the outbreaks that have occurred in North Dakota. Wilson (38) points out that the disease seems to be most prevalent during the winter in dry sections of country.

E. Species of the Genus Eimeria Affecting Cattle

Cattle coccidia were first seen by Zürn and named Eimeria zurnii Rivolta (1878). This species was described by Smith and Graybill (32) as being circular or oval in outline, with a wall of uniform thickness and with average dimensions of 18.6 u by 14.8 u. The sporoblasts when first formed were spherical in outline but the sporocysts become ovoid in shape with a thick cap at the pole. Two sporozoites are formed which completely fill the sporocyst, and there is no residual body.

The second type of oöcyst, more rarely seen by Smith and Graybill, is much larger, measuring 29.9 u by 19.9 u. It has a thick wall, often brownish in color, which becomes much thinner at the small pole. There is a definite residual body and two sporozoites found in the sporocyst. This species was found in Russia and named Eimeria smithi by Yakimoff and Galonzo (from Wilson (38)).

Becker and Frye (7) describe Eimeria ellipsoidalis as a new species affecting cattle. The predominating oöcysts were ellipsoidal in shape, almost never ovoid or round. The sporocysts contained a residual body and two sporozoites. The average

measurements of the oöcysts were 23.4 u by 15.9 u. Recent investigations have demonstrated that the oöcysts cannot be classified according to species by size alone; therefore the work of Becker and Frye is not entirely conclusive, as is the case with Bruce's description of Eimeria canadensis.

Wilson (38) described Eimeria cylindrica as being almost entirely cylindrical in shape. The oöcysts measure 23.3 u by 13.3 u. The sporocysts when fully developed contained two sporozoites and a residual body. Wilson found that this species developed and became infective after twenty-four hours incubation.

F. Physiologic Characteristics

1. Cross Infection Experiments

There have been many conflicting results recorded from cross infection experiments. Some of the earlier observers believed that bovine coccidiosis might be caused by Eimeria stiedae of the rabbit. Tyzzer (33) concluded in his work that the chicken coccidia is highly host specific, and he showed the absolute necessity of controlled experiments to prevent accidental infection. He was successful in infecting the turkey with chicken coccidia, getting a slight infection in two chickens with Eimeria dispersa of the pheasant. Bruce (11) concluded that bovine coccidia were entirely host specific. Andrews (3) obtained infections in both dogs and cats with Isospora felis and Isospora rivolta, but he concluded that the other species of coccidia of mammals were host specific. Biester and Murray (10) found that coccidia of swine origin were infective for chickens. Eimeria tenella of the chicken was not infectious for swine. They conclude as follows: "Sporulated oöcysts of ovine origin did not prove to be infectious for swine.

Sporulated swine oöcysts administered to milk-fed calves did not produce an infection. Sporulated oöcysts of bovine origin fed to pigs resulted in infection. Cultures of oöcysts made after one swine passage again appeared infectious for pigs and a calf." However, much of their work has been discredited by recent experimentation.

Wilson (38) was unable to produce an infection in swine with bovine coccidia. The pigs were shown to be susceptible by feeding them oöcysts of swine origin. No infection resulted from feeding bovine coccidia to goats. Many of the fallacies and ill-founded conclusions reported in the literature may result from one host acting as a carrier of oöcysts of an unrelated host species, or from the failure of the investigator to employ adequate precaution to avoid accidental infections or to recognize them when they occur. Tyzzer (34) points out the success of Krijgsman in infecting rabbits with Eimeria stiedae from the feces of rats that had been fed this organism.

2. Artificial Excystation

A number of workers have reported success in producing digestion of the coccidial oöcyst in vitro. Andrews (4) reported that he was able to bring about digestion of the oöcysts of dogs, cats, guinea pigs, pigs, and prairie dogs by feeding the oöcysts in milk to young rats. He was able to demonstrate sporozoites from the intestinal content one hour after feeding. He concluded that any species of coccidia might be digested by this method. This differs from the conclusions of a previous report (3) in which he stated that the oöcysts of a foreign host were so resistant to digestive action that they would pass through the host unchanged. Wilson (38) used pepsin and hydrochloric acid, trypsin, and sodium carbonate, pancreatin, and fresh bile in an unsuccessful attempt to digest bovine coccidia in vitro. A high percentage of the sporozoites of Eimeria of the chicken were liberated by this method. He fed bovine coccidia to young rats and killed them in one and two hours, but the oöcysts were unchanged. The same culture was fed to a young white rat with an illeac fistula, but there were no sporozoites liberated. In both cases Wilson was able to demonstrate the digestion and liberation of the sporozoites of chicken coccidia.

3. Effects of Temperature and Chemicals Upon the Oöcyst and Its Development

Until the appearance of two articles in 1931, very little was known concerning the effects of chemicals or temperature upon the oöcysts of any of the species of Eimeria. Wilson (38) refers to Pérard as stating that Eimeria perforans and Eimeria stiedae are resistant and withstand the ordinary disinfectants and acids. Pérard did not give the strengths of the solutions used. The oöcysts were susceptible to desiccation and were killed after an hour's exposure at 55°C, and eighty per cent were destroyed in twenty minutes at the same temperature.

According to Hutyra and Marek (21) a five per cent solution of the cresol compounds will destroy bovine coccidia.

Frank (18) found that bovine coccidia were not killed by drying or freezing. Fish (15) stated that the Eimeria of the chicken is not as resistant as is believed. They are highly resistant to chemicals, but they are very susceptible to physical agents. Both segmented and unsegmented oöcysts are killed at 55° C in ten minutes or at 60° C for twenty seconds. The oöcysts are not

resistant to ultra violet rays. Wilson (38) found that the winter temperatures encountered in Iowa destroyed both sporulated and non-sporulated oöcysts of the Eimeria of cattle. The maximum thermal death time of non-sporulated oöcysts is about 55° C with an exposure of ten minutes. He concludes as follows:

"Non-sporulated bovine coccidial oöcysts are readily destroyed by mercuric chloride, phenol, liquor cresolis compound, and formaldehyde, but were not destroyed when incubated in two per cent potassium dichromate or two per cent copper sulphate. Drying in the direct rays of the sun for twenty-four hours killed Eimeria oöcysts of bovine origin. Drying, out of the sun, was less effective."

4. Rate of Development

Although there are still certain phases in the life cycle of bovine coccidia to be studied, the rate of development outside the body of the host seems to be firmly established.

Degoix, according to Hutyra and Marek (21), found that bovine coccidia would sporulate in four or five days or remain in fecal material for two and a half months without losing their power of sporulation. Putrefactive changes lasting three months do not destroy the oöcyst. Becker and Frye (7) used one per cent potassium dichromate as a preservative and found that Eimeria smithi developed spores in two weeks, and that Eimeria ellipsoidallis was fully sporulated by eighteen days when kept at room temperature.

Biester and Murray (9) found that two per cent potassium dichromate, with one gram of bone charcoal for each 10 cc of culture, was best suited for the development of swine coccidia when kept at a temperature between 21° to 32° C. The cultures must be aerated daily to get maximum sporulation. They state that the depth of the medium does not appear to be a factor, provided the cultures are aerated.

Tyzzer (33) used 2.5 per cent potassium dichromate for the sporulation of avian coccidia. According to Tyzzer, oxygen is necessary for sporulation, not only a free access to air alone, but optimum conditions for development are obtained with potassium dichromate, which is an oxidizing reagent.

Fantham (14) found that when freshly voided droppings of grouse containing coccidial oöcysts were allowed to dry, the oöcysts in the surface layers rapidly developed sporocysts, the inner ones remained unchanged. He states, "Faeces kept en masse in covered dishes for as long as twelve months have retained the power of infecting birds, as I have been able to show experimentally. Such material contains undifferentiated oöcysts still, while its outer layers mainly contain oöcysts with four sporocysts within them." Fantham observed that the Eimeria of the grouse, when kept in water at 20° C developed in nine days. There was little degeneration before the fortieth day. Some of the oöcysts degenerated and others completed their development and their four sporocysts, apparently unharmed, were set free into the liquid. He concludes as follows: "The development of the oöcysts and sporocysts is delayed by the

presence of much moisture, but that the power of infection is retained for a long time by means of the sporocysts."

Wilson (38) used two per cent potassium dichromate for the sporulation of bovine coccidia. All cultures were kept at a temperature range of 27° to 30° C, and they were aerated daily from three to fifteen minutes by passing compressed oxygen through the cultures.

Table 1 of Wilson's shows a comparison of sporulation time for the four species of the genus Eimeria affecting cattle.

Name	Time for Sporulation
1. Eimeria zurnii	12-20 days
2. Eimeria smithi	14-21 days
3. Eimeria cylindrica	2-10 days
4. Eimeria ellipsoidalis	14-18 days

The life cycles of the Eimeria affecting cattle have not been studied in detail. Bruce (11) found that the period elapsing between infection and the first passage of blood or oöcysts in the fecal material was about two weeks. This agrees with the results of Roderick's (29) observations. Wilson (38) found that when fully sporulated oöcysts were fed to susceptible young calves, non-sporulated oöcysts appeared in the feces in

about seven days. Fish (17) believes that the body temperature, rather than the lack of oxygen, prevents the development of oöcysts in warm-blooded animals.

G. Resistance and Immunity

The term immunity is used in reference to coccidiosis to imply resistance in a general sense without regard to the nature of the protective reactions. Law (24) stated that suckling calves were immune. Tyzzer (33), working with the Eimeria of the chicken, found that certain breeds have a natural immunity. This observation agrees with that of Fantham (14). Tyzzer's results showed that the species of Eimeria that penetrated deeply in the intestinal mucosa excited a well-marked and prompt immunity; however, only species immunity was produced. Passive immunity could not be produced. A similar host response to coccidial infection in chickens was noted by Johnson (22), and Henry (20).

Fish (17) believes that immunity to coccidiosis in the chicken is comparatively slight and, in the majority of cases, is not sufficient to protect the host against subsequent infections. He thinks that the self limitation of the life cycle of the parasite, and the general condition of the bird, are of more importance than immunity.

Andrews (2) reports that an acute attack of coccidiosis in both dogs and cats will produce an immunity which will last for seven months, and possibly for life.

Immunity to coccidiosis in the rabbit was studied by Bachman (5). He found precipitins in high dilutions in rabbits artificially immunized, but precipitins were negative in rabbits which had been injected. Henry (19) states, "Intradermal injections of oocysts of E. caviae into guinea pigs which had recovered from infections with this coccidium clearly demonstrated a cutaneous hypersensitivity."

Wilson (38) was able to establish species immunity with the Eimeria of the bovine. He concludes: "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."

H. Influence of Diet

No knowledge exists concerning the nature of the influence of diet on bovine coccidiosis. According to Fish (17), Hegner (1924) and Ratcliff (1928, 1929), marked changes have been reported in Endamoeba, Chilomastix, and Trichomonas infections in the rat when the protein content of the diet was raised. Allen (1), working with chickens, concluded the birds on a high-protein diet and high-vitamin diet have a much lower mortality rate when infected with coccidia than chickens on a low-protein and low-vitamin diet. She states, "The presumption is that the high-protein or high-vitamin content of the diet is correlated with, and is directly or indirectly responsible for the escape from severe acute coccidiosis and the production of a prolonged coccidiosis. Possibly both proteins and vitamins are correlated with this result. The mechanism of the result may be regarded as a form of resistance producing immunity to acute coccidiosis, or resistance slowing the life cycle so that a predestined oöcyst's production is slowed up and stretched over a long period of time instead of being rapidly completed with production of acute clinical coccidiosis, or the resistance may involve both types."

I. Symptoms

The symptoms of bovine coccidiosis are characteristic and have been well described by numerous workers. Schultz (30) observed cases which showed a nasal discharge with some hemorrhage, an inflammatory condition of the eyes, and constipation, which was later followed by a diarrhea. The temperature ranged from 104° to 107° F. In some instances the cases terminated fatally in two or three days.

Lentz (25) stated that diarrhea was the first symptom shown. This changed to mucus, mixed with blood clots and feces. Way and Hagan (36) observed a bloody diarrhea as the first symptom, accompanied by a moderate rise in temperature and pulse rate. Barnes and Brueckner (6) reported on cases in calves which passed almost pure blood. There was some straining and frequent attempts were made to defecate. Bruce (11) gave a very good description of the symptoms shown in cattle suffering from coccidiosis. He stated that constipation might be the first symptom shown, followed by a diarrhea containing blood, mucus, and shreds of epithelial tissue. There was a rapid emaciation, abnormal appetite, and a grinding of teeth. Cerebral disturbances were present in some cases. There was usually little or no rise in temperature.

The reports of Frank (18), Marsh (26), Muldoon (27), and others bear out these observations.

Wilson (38) sums up the symptoms of the disease as follows: "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 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."

Becker and Frye (7) report on finding coccidial oöcysts in apparently healthy calves.

J. Pathology

The pathologic changes produced in bovine coccidiosis have been described by a number of workers. Hutyra and Marek (21) state that the small intestine is the principal site of the infection in calves, but that the infection is almost entirely confined to the large intestines in older animals. Wilson (38) states that Metzner was the first to point out that coccidia may invade the subepithelial tissue. Degoix (13) stated that bovine coccidia are never found in the mucous layer of the intestine but occur in the deeper-lying tissue and the glands of Lieberkuhn. Bruce (11) agrees that the glands of Lieberkuhn are one of the chief sites of the infection, but he states that the organism may be found in the intertubular cells. He found areas of erosin in the abomasum but no coccidia were present. This agrees with the findings of Way and Hagan (36), who stated that the lesions were confined mainly to the large intestine, and that the rectum was more involved than the colon or caecum.

Lentz (25) observed on postmortem that the mucous membranes of the large intestines were reddish-brown in color, and that the spongy tissue could be scraped away, leaving large superficial ulcers. Smith and Graybill (32) found that the infection was

usually confined to the large intestine, although occasionally a gland in the small intestine showed an invasion by the parasite. Pin-point hemorrhages also occurred; these tended to coalesce until there was a sloughing of the surface epithelium, followed by capillary hemorrhage and necrosis.

Jowett (23) found in one calf on postmortem that the lesions were confined mainly to the small intestine and that the inflammation was most pronounced in the duodenum.

Wilson (38) made a very careful study of the pathology. He reports that, "The pathologic changes in bovine coccidiosis are primarily confined to the intestines, principally the caecum, 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." He found that the parasite invaded the epithelial tissue of the deeper portions of the intestinal glands. The lesions were most abundant in the rectum.

He continues, "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."

III. Experimental

A. Purpose of Study

The reports in the literature within the last ten years have pointed out the prevalence and economic importance of bovine coccidiosis. The work of Tyzzer (33) with chicken coccidia has stimulated investigations and Wilson's (38) extensive study of bovine coccidiosis has opened up new fields for research. The knowledge existing previous to these two reports was based upon field observations and inconclusive laboratory experimentation. Great advancement has been made during the last two years in the study of the biology of the species of Eimeria affecting the chicken. The study of the phases of the disease in cattle is much more difficult because a large number of animals cannot be kept for laboratory experimentation under ideal conditions, except at great expense. For this reason, advancement in the study of bovine coccidiosis will be slow and it will be necessary to confine investigation and experimentation to certain definite phases of the disease. This investigation has been limited to a study of the physiologic characteristics of the species of Eimeria affecting cattle, with a general consideration of the disease.

B. Material and Methods

During the course of this experiment nineteen calves, one cow, four lambs, and a number of white rats have been used. The experimental calves, either Jersey or Holstein, were obtained from the college dairy herd, when they were less than a week old, and were kept under observation before being placed on experiment. They were confined in a large, well ventilated and well heated room which was neither rat proof nor fly proof. Three calves at a time were used. Their quarters were separate cages made of steel mesh, with solid steel walls, giving them no chance to communicate with each other. The cages and their removable wooden floors were cleaned daily with a hose. The cement floor of the room was equipped with a large drain to carry off all fecal material. The feeding troughs were hooked to the doors, and they were removed after the animals were fed, to prevent contamination with fecal material. When the calves were changed the cages and floors were thoroughly cleaned and disinfected with high pressure steam. The calves not being used were kept in individual wooden pens or stanchions.

At first the animals were fed skim milk, and later chopped alfalfa hay and a 24 per cent dairy ration, in the proper amounts. Green grass was given regularly, except during two or three winter

months. This ration gave good results; the calves grew and gained as much as could be expected in such close confinement. The milk was fed in individual pails which were washed and steamed after each feeding. No attempt was made to sterilize the feed because it was considered impractical and the possibilities of accidental infection, if properly handled, were believed to be almost negative. Two or three non-infected susceptible control animals were kept at all times in the same room with the infected calves. At no time during the course of the experiment was there a case of accidental infection in the control calves.

The lambs were purchased when they were almost two weeks old. These were orphan lambs which had been running in a chicken lot on a farm where no sheep had been kept for years. They were confined in individual wooden cages with a slat floor, in an isolated room. The room was not rat proof, but it was fly proof. No one entered the room except the attendants, who fed the lambs and cleaned the drop pans under the cages. The lambs were bottle fed on pasteurized milk. The nipples and bottles were sterilized between each feeding with boiling water and high pressure steam.

Daily fecal examinations were run on all calves for at least two weeks after they were obtained. Calves that were not being used on the experiment were examined three times a week. Only two calves were

found to be passing coccidia at the time they were secured. These two passed an occasional coccidium, but they soon became negative. The sugar flotation method, described by Benbrook (8), was followed in making fecal examinations. Fecal samples were obtained for all examinations by dilating the anus and rectum, thus insuring a fresh representative sample.

The oöcysts were incubated in either 1.5 per cent potassium dichromate or 2 per cent copper sulphate solution in a room ranging in temperature from 20° C to 25° C. Half gallon jars containing three pints of culture material were used to sporulate the oöcysts. The cultures were aerated once or twice a day from three to five minutes by forcing air into the bottom of the solution with a pressure bulb. Pint milk bottles with cork stoppers to prevent evaporation were used when there was not a large amount of material to be sporulated. The bottles were shaken two or three times daily, and the corks were removed at intervals to change the air. Flat bottomed porcelain pans were used when maximum sporulation was desired. The cultures were prepared by adding equal amounts of copper sulphate or potassium dichromate to a suspension of fecal material which had been screened to remove organic and coarse fecal material. Water was added to make up for the loss by evaporation. The potassium dichromate was removed from the cultures before feeding by dialyzing the cultures

from 48 to 72 hours.

The cultures used in this experiment were obtained from various cases of coccidiosis. Culture A was obtained from the intestinal content of a yearling bull which came to necropsy as a result of an acute coccidial infection. From microscopic examination the culture appeared to contain about 90 per cent Eimeria zurnii and 10 per cent Eimeria smithi. Culture B came from an experimental case of coccidiosis in an Iowa calf. Culture C was obtained in Iowa and contained only Eimeria smithi. Culture D was sent to the laboratory for examination from Appomatox, Virginia. Microscopic examination showed it to be a very rich culture and all of the oöcysts conformed to the morphologic description of Eimeria zurnii. Culture E was collected from six calves in Grayson county, Virginia, suffering from an acute attack of bovine coccidiosis. This culture was later shown to contain Eimeria zurnii, Eimeria cylindrica, and Eimeria smithi. Culture F, containing oöcysts of Eimeria zurnii predominantly was from a case of coccidiosis in Blacksburg, Virginia. Culture G came from a field case of coccidiosis in Iowa, and was obtained from the rectum of a cow with an acute attack of bloody diarrhea. Culture H, of ovine origin, was obtained from experimental lambs kept in the laboratory. This was a rich culture and appeared to have oöcysts of three distinct size ranges.

C. Source of the Infection

The histories in some of the field cases observed during this investigation have suggested certain possibilities and indefinite conclusions, which have had a marked influence on the course of this experiment.

In November, 1931, a 1000 pound yearling bull, which died as a result of an acute coccidial infection, was brought to the laboratory for a post mortem examination. Death had resulted about a week after the onset of the first symptom and before marked emaciation had been noted. The lesions presented were typical of a severe acute infection. Diffuse hemorrhages covered the entire surface of the rectum, colon, caecum, and posterior part of the illeum. Areas throughout the colon and rectum were entirely denuded of surface epithelium. The posterior part of the intestines contained hemorrhagic fluid, shreds of epithelial tissue, and large organized blood clots with a diameter of about two inches. Oöcysts were found in abundance and a very rich culture designated as culture A was obtained from the intestinal content. The diagnosis was further confirmed by a histologic examination. This was the first case of bovine coccidiosis observed by the owner, but he was positive that no animal on his farm had ever shown symptoms of the disease before. All the animals on the farm were old; there had been no additions made to the herd for several years; the bull seldom came in

contact with other animals, so the source of the infection could not be explained.

Several writers have pointed out that the disease seems even more prevalent in certain districts in which the source of the infection cannot be determined. There is the possibility of immune animals or carriers acting as spreaders of the infection. In coccidiosis the term "carrier" is used to designate only those animals which eliminate oöcysts without showing clinical symptoms of the disease. The organism might be considered more or less as a normal inhabitant of the intestinal tract of these individuals. Robertson (28) believes that carriers or tolerants are the main sources of the infective organisms. Roderick (29) was unable to find oöcysts in the feces of cattle, either in healthy or affected herds, which did not present clinical symptoms of coccidiosis. Becker and Frye (7), and other workers, have reported finding oöcysts in apparently healthy animals. It has been observed that apparently healthy cattle in both infected and non-infected herds may occasionally pass such small numbers of oöcysts that the organism may pass unnoticed in routine fecal examination. If the carrier, or tolerant, is responsible for the spread of the infection, it is at once apparent that it is impossible for the susceptible host to ingest a large infective dose at any one time. This assumption may be reasonably reached without

taking into consideration the percentage of sporulation attained by the organism under natural conditions. The evidence in the literature seems to support the theory that the infection is spread through the tolerance of immune animals. Otherwise the unfavorable conditions encountered in nature would soon destroy the organisms and the disease would disappear. Wilson (58) has shown that sunlight, drying, putrefaction, and winter temperature as encountered in Iowa, destroyed bovine coccidia. Wilson collected a culture of bovine coccidia in March, 1930, from an Iowa pasture on which no animals except cattle had ranged, and the pasture had been vacant since November, 1929. Only non-sporulated oöcysts were found, and these were not present in great numbers. It seems that only under the most favorable circumstances does the organism withstand the combined destructive actions of nature.

The assertion that the natural infective dose of the organism is very small is further supported by the observation of two cases of acute coccidiosis in month old calves in the college dairy herd. Three calves were confined in a 10 x 12 foot concrete stall, bedded with straw. Grain which had been mixed in the feed room was fed in troughs outside the stanchions, and milk was given from individual pails. The stalls were cleaned once a week, the troughs cleaned before each feeding, and the pails were sterilized with flowing steam between feedings. These calves had never been out of the stall. One died as a result of acute

hemorrhagic enteritis four days after the onset of the diarrhea. The remaining two calves were moved and the stall was thoroughly cleaned.

Three young calves were placed in the same stall a month later. On February 4, 1932, one of these calves developed a diarrhea with an odor characteristic of intestinal hemorrhage. The next day the animal began passing shreds of epithelial tissue and a mixture of blood and mucus. The third day after the onset of the first clinical symptom, hemorrhage was profuse and the animal was killed for a post mortem examination. Hemorrhagic areas were found throughout the rectum, colon, caecum, illeum, and posterior part of jejunum. Histologic examination showed extensive lesions in the rectum, colon, caecum, and illeo-caecal valve. There were occasional lesions found in the terminal portion of the illeum. A pure culture of Eimeria zurnii, known as culture F, was collected from the intestinal content.

In attempting to discover the source of the infection, routine fecal examinations were run on all the calves in the barn, and litter from all the stalls was examined thoroughly for oöcysts. All but the two calves confined in the infected stall were negative for coccidia on this examination and on repeated ones. These two animals were passing occasional oöcysts in which the cytoplasm had shrunk, and it

was concluded that these oöcysts were picked up from the bedding. The two calves were moved to new quarters and the discharge of oöcysts stopped almost immediately, and no clinical symptoms developed later.

Straw and litter, scraped from the floors of each stall, was placed in buckets with three quarts of water added to each one. The buckets were shaken thoroughly; the water was drained off and centrifuge samples were run for each specimen. No coccidia were found except from the stall where the infection had occurred. The coccidia were found to be numerous in the material taken from this stall. The contrifuged sample gave an average count of twelve oöcysts per field under a high power of the microscope. No sporulated oöcysts could be found.

Two non-infected, susceptible calves, three weeks old, were placed in the infected stall in an attempt to reproduce natural infection. The grain and hay were put on the floor in various places in the stall, making it possible for the calves to pick up a larger number of oöcysts than they would have if the feed had been put in the trough. It was hoped that natural infection would occur and that oöcyst production and the resulting pathogenicity could be studied. The calves remained in this stall for three weeks and daily fecal examinations were made. From the second day there was a continuous

passage of small numbers of degenerated and stained oöcysts of Eimeria zurnii in the fecal discharge. A weekly examination of the litter in the stall was made, but only non-sporulated oöcysts could be found. After a period of three weeks the animals were moved from this stall to the laboratory. The fecal examinations became negative for coccidia five days later, and remained negative until the animals were put on pasture.

The manner of feeding and the concentration of the oöcysts provided optimum conditions for infection, but it is apparent that no infective forms of the organisms developed. On range or pasture there would be even less favorable conditions for infection. It is apparent that natural infection is brought about by a more or less continuous ingestion of small numbers of coccidia, of which a very small percentage of oöcysts must necessarily be sporulated.

D. Pathogenesis following Artificial Infection

1. Natural Resistance

A number of workers have had difficulty in reproducing coccidial infections experimentally. Roderick (29) suggests that some accessory, or predisposing, factors are involved in addition to the ingestion of ripened oöcysts. Tyzzer (38), Fantham (14), and others have noted that certain breeds of chickens seem to be more resistant to coccidial infection than others. There is no evidence in the literature studied suggesting a similar natural immunity in different breeds of cattle. In this experiment, Jersey calves seemed to be more susceptible to artificial infection than Holstein calves, but there were exceptions in both breeds. More Holsteins than Jerseys were used. They adapted themselves to their environment and grew faster than the Jerseys. The variations noted probably resulted from individual resistance rather than racial immunity. Two calves showed a natural immunity.

Calf No. 1, when it was two weeks old, was drenched with 500 cc of sub-culture A. Ten days later the calf developed a severe diarrhea, which ceased four days later. No oocysts were found in the fecal discharge until the twentieth day. A careful examination of the slide from a centrifuged sample only revealed two oöcysts of Eimeria zurnii. Fecal samples continued to be negative for oöcysts for a period of 24 days following, at which time the examinations were discontinued.

Calf No. 2 was given 1000 cc of mixed sub-cultures of culture A by stomach tube. Degenerated and stained oöcysts were passed on the fourth and fifth day. Fecal examinations were negative from the time of feeding until thirty-four days later. The calf was then drenched with 1400 cc of sub-culture of culture B. This was a very rich culture in which Eimeria zurnii predominated. On the seventh day a severe diarrhea developed and on the following day the fecal discharge consisted mainly of mucus with an offensive odor. Only occasional oöcysts were passed on the nineteenth and twentieth days, but on the following day the average count of oöcysts per low power field was six Eimeria zurnii and two Eimeria smithi. The fecal examinations were negative until the thirty-third day, when the calf passed a small number of oöcysts of Eimeria smithi. Fecal examinations were discontinued.

The results obtained with these two calves demonstrate a natural resistance. The animals showed a slight susceptibility by the passage of a limited number of oöcysts, but the slight infection produced was not in proportion to the massive doses of infective material fed. There was no possibility of a tolerance having been developed, because the fecal discharges of the calves had been negative for coccidia from the time of birth.

2. Acquired Immunity

Coccidial infection in calves apparently confers an immunity of varying degrees against subsequent infections. The type or duration of immunity conferred by one infection is unknown. Robertson (28) believes that the rareness of infection in older animals is a result of tolerance, or an immunity developed from infection early in life. Tyzzer (33), and others, found that a well marked and prompt immunity was established in the chicken by coccidiosis, especially from the species which penetrated deeply into the tissues. A well marked immunity resulted in two calves from severe infections. In one of these cases the immunity was known to have endured for at least five months.

Calf No. 3, when three months old, was fed 1200 cc of culture B. Twelve days later there was a sudden onset of a fetid diarrhea, streaked with mucus and blood, and oöcysts appeared in great numbers. The discharge of oöcysts continued from the twelfth to the twenty-third day. About 95 per cent of the total oöcysts were Eimeria zurnii and the remaining 5 per cent were Eimeria smithi. The number of oöcysts passed gradually dwindled and the examinations were negative from the thirty-first to the forty-first day.

Four days later a second dose of 200 cc of culture A was administered. The calf remained negative on fecal examinations, and one month later the third dose, consisting of 300 cc of culture A, was

fed. Fecal examinations were made daily for a month and a half, but no oöcysts were found. Five months after the primary dose was given, the calf was fed 1500 cc of an exceedingly rich sub-culture of culture B. No infection resulted, as was shown by repeated microscopic examinations of the fecal discharge. Calf No. 4 was fed 1000 cc of a mixture of cultures A and B. The passage of oöcysts started on the seventh day and continued until the thirteenth day. During this period there was a diarrhea of a watery consistency except on the ninth and tenth days. Then there was profuse hemorrhage with a passage of mucus and long shreds of epithelial tissue. The average count during the peak of the oöcyst production was 30 oocysts of Eimeria zurnii per high power field. The second crop of oöcysts was passed from the eighteenth to the thirty-second day, continuing over a much longer period than the first, but the oöcysts were not as numerous. They were predominantly Eimeria zurnii and occasionally small numbers of Eimeria smithi. The calf had a diarrhea throughout the second period, but there was no perceptible hemorrhage. Appetite remained fair to good, with a few exceptions. Fecal examinations remained negative after the thirty-third day.

Two months after feeding the first infective dose, the calf was dosed with a 1000 cc of culture material collected during the first passage of oöcysts. No infection resulted.

Primary infection in the above two cases protected the calves against the ensuing infective doses given.

Species immunity is considered to be specific in calves as it is in chickens. Wilson (38) was able to produce species immunity in calves and could isolate pure cultures of coccidia by specific immunization. An attempt was made to establish in two calves a specific immunity for Eimeria smithi.

Calf No. 5. This animal was drenched with 400 cc of culture C, which was known to be a pure culture of Eimeria smithi. This was a very lean culture; only five or six oöcysts from an examination of a centrifuged sample were found. On the ninth day a diarrhea with a characteristic odor of intestinal hemorrhage developed. On the day following two oöcysts were found in a centrifuged sample. On the seventeenth day the diarrhea again became marked and oöcysts were passed for four days, the maximum count averaging 20 per low power field. Fecal examinations were negative for a week. Oöcyst production started again on the thirty-first day and lasted eight days. An average oöcyst count showed the maximum number passed at any one time to be 20. On the fifty-third day 1200 cc of culture D was given in a drench. This was an exceedingly rich culture of Eimeria zurnii. Two oöcysts of this species were found in a centrifuged sample on the tenth day. A slight diarrhea was present at this time but no infection resulted. This calf was drenched on the seventy-second day of the experiment with 800 cc of culture E (mixed culture). Occasional oöcysts of Eimeria smithi were passed but no infection resulted, and the experiment was discontinued in a month.

Calf No. 6 was drenched with 600 cc of a sub-culture of culture E. The sub-culture had been sporulated in 2 per cent copper sulphate. A diarrhea streaked with hemorrhage developed on the eleventh day. Oöcyst production started on the twelfth day and the oöcysts of Eimeria smithi were found in great numbers for a period of 13 days. Fecal examinations remained negative, and a month later 200 cc of culture E was given. The calf had a foul smelling, watery diarrhea until this time. Although occasional oöcysts of Eimeria zurnii and Eimeria smithi were found in the feces, no marked infection developed.

The results of these experiments would indicate a cross immunity rather than a species immunity. As previous studies indicate immunity is a local or cellular reaction rather than a seriological reaction. It seems probable, therefore, that infection of calves with one species of coccidia would protect the animals against infection of another species, provided the site of the infections were the same. A variation in the site of the infection might explain the reported success of species immunization in calves. A mild infection with one species does not always protect the animal against subsequent infections with the same species. In the preceding experiments a heavy infection with Eimeria smithi failed to provide complete protection against a mild infection later with the same organism.

An animal's resistance and tolerance probably increases with its age. Active immunity is probably maintained during the life of the animal by the

continued ingestion of small numbers of coccidia. The possibility of overcoming the resistance in an old animal by feeding a massive dose of infective material was tested in one cow.

Cow No. 1. This eleven year old animal was obtained from the college dairy herd. The cow was drenched with 2000 cc of culture E. This animal was negative for Eimeria oöcysts when the culture was administered. Stained and degenerated oöcysts were passed on the fourth day. From the sixth to the eleventh day large numbers of epithelial cells were found on microscopic examination of the feces. No infection developed, and the examinations were discontinued a month later.

Immunity in this animal was probably conferred by a mild infection during early life.

3. Other Factors

a. Dosage

The number of infective oöcysts fed to a susceptible calf is not a criterion of the degree of the infection produced. Fish (15), in his work with Eimeria of the chicken, believes that there is no correlation between the size of the infective dose and the height and duration of the patent period. The most severe infections were obtained in two cases by feeding very small numbers of coccidia. The response to massive doses varied from a mild to a well marked infection. From careful study of the symptoms and the production of oöcystsin experimental calves, it becomes apparent that there is no correlation between the dosage and the manifested infection. Other factors seem to play a more important part in coccidial infections than dosage. This assumption is further borne out by the observation of a number of field cases of coccidiosis.

B. Attenuation of Oöcysts

In the experimental infection of nineteen calves there were no resulting mortalities. The cases of artificial infection did not assume the seriousness of any of the cases of natural infection observed. The difficulty experienced in producing a well marked infection by feeding massive infective doses suggested the possibility of some necessary predisposing factors. Although it would be impossible in experimentally induced laboratory infections to reduplicate all of the factors encountered in natural coccidial infections, it might be possible to reproduce some of them. The most obvious difference between artificial and natural infections is the manner in which the oöcyst is sporulated. The question of whether sporulation of the oöcyst in an oxidizing reagent, such as potassium dichromate, might attenuate the parasite to some extent suggested itself. An attempt was made to reproduce conditions for natural sporulation by placing the oöcysts in a soil culture. Two quarts of culture E were screened three times to remove organic material and added to an equal amount of sterilized soil. This was placed in a large flat bottomed porcelain pan, and enough distilled water was added to keep the culture moist at all times. The culture was aerated daily by a thorough mixing. No sporulated oöcysts could be found until a month later. A smear made from a centrifuged sample of this material revealed only one or two sporulated organisms, although there was an abundance of non-sporulated ones. Further development failed to take

place and it was decided to test the viability of the segmented oöcysts and their ability to produce infection by feeding a large quantity of this soil culture to a susceptible calf.

Calf No. 7. A quart of soil culture was repeatedly screened to remove the coarse particles of dirt. This material was given to the calf in small quantities and washed down with water. A mild diarrhea began on the ninth day and continued until the twenty-third when it became severe. Straining was marked and numerous attempts were made to defecate. There was a hemorrhagic diarrhea with a foul odor. Marked symptoms of the disease were shown by the calf throughout the course of this experiment. Emaciation of the animal was noted on the sixteenth day, and it became so marked eight days later that the outline of the skeleton was visable. The animal was so weak during this period that it was unable to stand and the animal was not expected to live. The eyes became sunken, the coat was dull, and there was a continual grinding of the teeth. There was no evidence of a secondary infection because the temperature remained normal and the animal retained some desire to eat. A nephritis was manifested by the passage of bloody urine from the eighteenth to the twentieth day. The passage of oöcysts continued over a long period. Small numbers of Eimeria smithi were passed in pure culture from the ninth to the seventeenth day. During the ensuing ten days large numbers of E. zurnii, E. smithi, and E. cylindrica were passed. After this passage the fecal passages became normal and the animal made a rapid recovery in weight. Another period of diarrhea began on the

fiftieth day and small number of E. smithi were passed for five days. On the sixtieth day oöcysts of an unidentified species were passed in small numbers. The average measurements for seven oöcysts were 42.6 u by 25.2 u. The feces became negative for oöcysts and the experiment was discontinued. This calf presents a picture of a typical parasitological condition. Although no definite conclusions can be drawn, this experiment indicates a possible difference between the virulence of oöcysts sporulating under natural conditions and those sporulated by artificial methods. If future experiments verified this theory, it would be of considerable economic importance in preventing losses from coccidiosis. An immunity might be developed in calves by feeding small amounts of oöcysts sporulated in potassium dichromate.

With this in mind, it was decided to test the effects of potassium dichromate cultures on farm calves. Five calves in Grayson county, Virginia, were dosed with 500 cc of culture E. Culture E had been collected from six calves on the same farm in a previous outbreak of coccidiosis. This was the first outbreak of the disease. The healthy and infected calves had always been in separate pastures. These five calves were negative for Eimeria oöcysts before they were dosed with infective material. The owner was instructed to watch the calves carefully for symptoms. If the slightest diarrhea was manifested, he was to send fecal samples to the laboratory. No symptoms of the disease developed and the calves have remained healthy for five months. Fecal

samples collected from the calves showed a mild infection with Eimeria smithi, and also a much larger oöcyst. The oöcysts of Eimeria smithi measured 29.8 u by 19.8 u, while the larger oöcysts measured 46.8 u by 21.6 u.

Culture E fed to these calves did not produce marked infection, but it did produce a mild one which might result in protective immunity. No definite conclusions could be drawn from this experiment, but the suggested possibilities were that there was either an active immunity in these calves, or that the oöcysts fed had been attenuated to some extent by sporulating them in potassium dichromate. To check this it will be necessary to dose a large number of calves shortly after birth and before an active immunity can be developed.

C. Self-limitation of the Parasite

Tyzzer (33) concludes that coccidiosis in Gallinaceous birds is a self-limited infection; its duration in the absence of infection depends primarily upon its cycle of development, rather than upon the protective reaction of the host. In a recent article he (35) calls attention to the occurrence of great numbers of merozoites in the intestinal discharge, and this is unfavorable to the coccidium, in that its chances of propagation are diminished. Wilson (38) believes that possible 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 parasite. Clinical study of the Eimeria infections in calves based upon the elimination of the oöcysts indicates that the resistance of the animal has a marked influence in determining whether development of the merozoites will be sexual or asexual. Histologic study has shown that schizonts with daughter nuclei are developed within four days.

Calf No. 8 was fed 400 cc of culture E and killed for a post mortem examination, and 196 hours later macroscopic examination showed small patches of petechial hemorrhages throughout the rectum, colon, and caecum. Centrifuged samples of the intestinal content from the different parts were all negative for Eimeria oöcysts, with one exception. A smear made from a centrifuged sample of caecal material contained two oöcysts of Eimeria zurnii and three of Eimeria smithi.

These oöcysts showed a marked contraction of the cytoplasm, and they were believed to have been retained by the calf from the original infective dose. No sporozoites or merozoites could be found. Histologic sections showed only schizont formation with daughter nuclei.

It is probable that the first crop of merozoites are liberated about the fifth day, although none have been found in the fecal discharges. In a great many of the experimental calves a marked diarrhea has been noted on the fifth day or soon afterward. It would be possible, as Tyzzer suggests, for a large number of merozoites to pass out in the feces. This would greatly decrease the number of oöcysts resulting from the infection. Merozoites in the colon and rectum would tend to be lost in this way, and those retained in the caecum would be responsible for the continued infection. This might explain the lack of noticeable hemorrhage in mild infections. If the animal's resistance was marked, the majority of the organisms might become sexually differentiated after the first asexual cycle, and great numbers of oöcysts would appear in the feces between the seventh and tenth days. In other cases a greater number of organisms continue asexual development, and only a few oöcysts are eliminated, until active immunity brings about an elimination of the parasites by influencing either a gradual or marked sexual development. The results obtained in this experiment do not indicate a cyclical character of development with

a limited number of asexual generations for any of the species of Eimeria affecting cattle, but that the influence of resistance or immunity of the host is responsible for the apparent self-limitation of the parasite.

E. RESULTS

1. Cross Infection Experiments

Much contradictory evidence has appeared in the literature concerning the results of cross infection experiments. Recent experimentation has shown that coccidia of birds and mammals are highly host specific. Wilson (38) has shown that pigs are not susceptible to infection with the Eimeria of cattle. Goats fed coccidia of bovine origin apparently did not become infected. This experiment was not entirely satisfactory, as the goats used were not entirely free from coccidia.

The possibility of sheep being susceptible to infection with cattle coccidia was suggested by an outbreak of coccidiosis in lambs kept in the same laboratory with infected calves. These lambs when purchased at two weeks of age were free from all parasites. They were isolated in individual pens and were fed pasteurized milk. A month later fecal examinations showed that a majority of the animals were heavily infected with coccidia, although no symptoms of the disease were manifested. There were two explanations of the source of infection: first, that there was one undetected carrier, or second, that the lambs were infected with cattle coccidia carried from the infected calves by mice or flies. This latter explanation seems highly possible because the morphologic characteristics of the oöcysts passed did not conform to those of the described ovine species, but the oöcysts in some respects closely resembled certain species of bovine coccidia.

Four lambs were purchased soon after for a cross infection Experiment. They were isolated in wooden cages in a room which was fly proof but not rat proof. Every possible precaution was taken to prevent accidental infection. Daily fecal examinations over a period of two weeks were negative for coccidia.

Lamb No. 1 was given 200 cc of culture E. A diarrhea developed two days later and the elimination of oöcysts started on the fifth day. Oöcyst production became marked on the seventh day and continued for a week.

Lamb No. 2 was given 200 cc of a pure culture of E. smithi sporulated in 2 per cent solution of copper sulphate. The lamb began to eliminate oöcysts in small numbers on the fourth day. Between the seventh and the twelfth days the oöcysts passed were too numerous to be counted. The feces became negative for Eimeria four days later.

Lamb No. 3 became accidentally infected and began to eliminate oöcysts on the eighth day after being placed on the experiment, while the remaining control continued to be negative.

The morphologic characteristics of the oöcysts passed by the three lambs were the same. Three distinct size ranges were noted. Large oöcysts, either ovid or round, measuring 28.4 u by 21.6 u, were found in small numbers. The predominating oöcysts were cylindrical in shape and measured 25.2 u by 18 u. Smaller cylindrical shaped oöcysts measuring 19.8 u by 16.2 u were numerous.

No distinct polar cap or micropyle were noted, except in rare cases. The oöcysts sporulated in 24 to 48 hours. The sporocysts in all cases were elongated, cylindrical in shape, and contained a definite residual body. The oöcysts passed by these lambs did not conform to the descriptions given for either Eimeria faurei or Eimeria intrica.

Lamb No. 3 was killed for post mortem examination three days after oöcyst elimination started. No macroscopic lesions could be found. Fecal material was collected in separate containers from the different parts of the intestine, and examined for coccidia. The centrifuged samples revealed innumerable oöcysts in the material collected from the caecum and colon. Only one lesion of intra-cellular invasion could be found from histologic study; this was in a section of the illeo-caecal valve. The lesions studied showed only an invasion of the surface epithelium. Sections from the caecum showed a marked loss of surface epithelium with a noteworthy absence of hyperemia. This might explain the presence of enormous numbers of epithelial cells in fecal samples taken from infected lambs.

The rapid elimination of a great many oöcysts indicates that the infection in lambs is self-limited. The infection is characterized by the invasion of the superficial epithelium, principally of the caecum, with no apparent symptoms resulting.

Calf No. 9 was drenched with 500 cc of a sporulated culture collected from infected lambs. On the second and third day the calf passed a number of degenerated, non-sporulated oöcysts, but no sporulated oöcysts were found.

Elimination of oöcysts began on the eighth day, and a large number were passed on the ninth day. The diarrhea , which became marked at this time, had an odor characteristic of intestinal hemorrhage, and consisted mainly of mucus and epithelial cells. The fecal examinations became negative a short time later. About 95 per cent of the oöcysts passed were quite large, measuring 28.8 u by 14.4 u, and were distinctly cylindrical in shape. No micropyle or polar cap could be seen. A smaller one measuring 21.6 u by 16.2 u closely resembled E. cylindrica. Large ovid shaped oöcysts, measuring 30 u by 20 u were found. These were thought to be E. smithi. The oöcysts sporulated within 48 hours. The sporocysts were all cylindrical in shape and contained a residual body.

In view of the fact that no cases of accidental infection had occurred in calves during the course of this experiment, it was concluded that the infection resulted from the material fed. This is further supported by the fact that oöcysts did not appear in the feces until eight days after the infective dose was given. However, it is highly possible that the coccidial culture obtained from the infected lambs contained a number of oöcysts of bovine origin.

Although no conclusions can be drawn from the results of the cross infection experiments with lambs, a number of things are to be learned. It is clearly shown how easily accidental coccidial infection may occur in lambs. Experimentation with this disease can be carried on only under the most rigidly controlled conditions. The oöcysts encountered in this experiment

developed in 48 hours under favorable conditions. This would tend to cause accidental infections and lead to confusion in experimental work. From the morphologic study of the oöcysts passed it was believed that the infection was caused by a new, undescribed species of ovine coccidia. Even though most mammalian coccidia are highly host specific, there still remains a slight possibility that identical oöcysts, either of bovine or ovine origin, caused the infection in the lambs and in the calf. This might be the case in two animals such as the lamb and calf, which have such a close anatomical and physiological relationship. It is highly important to know whether the coccidia of sheep and cattle are entirely host specific. In view of the fact that it is impossible to interpret the results obtained from the foregoing experiments, and because of so wide a discrepancy in accounts of established cross infection, it would seem important for this subject to be reinvestigated to establish the facts of the case.

2. Effects of Temperature

The maximum thermal death time for bovine coccidia was established by Wilson (38). His results closely agree with those of other investigators. Other questions concerning the effects of a minimum temperature have not been answered.

Two hundred cc of culture E were placed in the unit of the General Electric refrigerator at -5° C. It remained frozen from January 5, 1932, until March 3, 1932. Microscopic examination showed that there had been a slight decrease in the number of oöcysts, proved by finding the shells of the degenerated oöcysts. Equal amounts of 1.5 per cent potassium dichromate were added to the culture when it was placed at room temperature. The culture was aerated daily for eleven days; an examination showed that 95 per cent of the oöcysts had sporulated. The culture was returned to -5° C on March 15, 1932. It was taken from the refrigerator on April 30, 1932, and examined. No oöcysts could be found in the culture. This culture was five months old at the termination of this experiment and the time element probably played a part in the destruction of the oöcysts.

A portion of culture E was sporulated and washed free from the potassium dichromate. This was exposed to outdoor winter temperature for five days. The culture was allowed to melt at room temperature and examined. There was no perceptible destruction of the oöcysts. It was placed at -5° C on March 15, 1932, and removed April 30, 1932. It was estimated that about 50 per cent of the oöcysts had been broken up. Exposure at a low temperature in this case

was not as long as in the preceding experiment. This culture was also free from potassium dichromate.

A sporulated sample of culture E was exposed to winter temperature from January 5, 1932, to April 30, 1932. A pint fruit jar was used as a container. The preservative had been removed from the culture by 72 hours dialization. Examination on May 1, 1932, showed that there was an approximate 50 per cent decrease in the number of oöcysts.

A non-sporulated portion of culture E was exposed under the same conditions and for the same period. It was estimated that about 25 per cent of the total number of oöcysts had been destroyed by the exposure. An equal amount of 1.5 per cent potassium dichromate was added and the culture was aerated daily for ten days. About 80 per cent of the oöcysts segmented during this time. It would seem that under favorable conditions bovine *coccidia would not be killed by exposure to a Virginia winter.* Non-sporulated oöcysts are apparently more resistant to a low temperature than the segmented forms. Increasing age probably causes a proportional death of a number of oöcysts. It remains to be seen what minimum temperature is necessary to destroy oöcysts by a limited exposure.

3. Age and Viability

No evidence can be found to show how long bovine coccidia will endure and retain their infective properties. Most investigators probably consider the average life of their cultures to be about five months. It has been noted during the course of this experiment that most of the sporulated oöcysts disappear from a culture in six or seven months.

The following experiment shows that E. smithi retains its power of sporulation and infectiveness for eighteen months.

Culture C, a pure strain of E. smithi, was collected from an infected calf in Iowa on May 26, 1930. It was preserved in 2 per cent potassium dichromate and aerated daily until sporulation had resulted. It was stored at 22° C until June 10, 1930, and shipped to V. P. I. The culture was examined June 25, 1932, but no Eimeria oöcysts could be observed microscopical and what appeared to be oöcyst shells could be seen. A large amount of this culture was dialyzed and two calves were fed this material. A three month's old calf received 1000 cc and the other calf, eight days old, was given 300 cc. No infection resulted in either animal. The remainder of culture C was returned to storage. When it was again examined on October 8, 1931, it contained ice from having been behind the unit of the electric refrigerator. Microscopic examination revealed the fact that a very small number of non-sporulated oöcysts of E. smithi had been previously overlooked. These oöcysts had been considered as infertile when the original culture had been sporulated.

Fresh preservative was added and the organisms were aerated three or four times daily. Sporulation was completed by October 28, 1931, and 400 cc of this material, which contained only about five oöcysts in a centrifuged sample, was given to a susceptible calf on November 14, 1931. A marked infection resulted and a pure culture of E. smithi was recovered. Refer to calf No. 5.

This culture contained both sporulated and non-sporulated oöcysts when it was first stored, but the sporulated oöcysts had disappeared after a year. It was clearly shown that the material had lost its infective power after the destruction of the segmented oöcysts. It is apparent from this experiment that non-sporulated oöcysts are more resistant than the sporulated form of the organism.

4. Effects of Disinfectants

Two cultures, A and E, which contained oöcysts of E. zurnii, E. smithi, and E. cylindrica were used for this experiment. The freshly collected fecal material was screened three times through wire mesh to break up large particles and remove organic material. After the oöcysts were exposed to the action of the disinfectants, as indicated in table 1, it was removed by washing four times in distilled water and centrifuging. The cultures were incubated in 1.5 per cent potassium dichromate. Large test tubes, about three-fourths full of culture, and tightly corked, were used as containers. The corks were removed for a short time, the tubes were aerated twice a day by shaking thoroughly, and the tubes were then placed in a horizontal position to give a maximum amount of surface. The tests were conducted for periods of three weeks, which allowed ample time for sporulation. The percentage of sporulation was based on a count of 100 oöcysts from centrifuged samples. Table 1 represents the results of two trials.

Table 1. Effects of Disinfectants

Time of Exposure	48 hours			1 hour			10 minutes		
Phenol	.5%	2.5%	5%	1.0%	2.0%	5.0%	2.5%	5.0%	10%
% Sporulated	0	0	0	72%	0	0	0	0	0
Mercuric Chloride	0.1%	0.2%	0.3%	0.1%	0.2%	0.3%	0.3%	0.5%	1%
% Sporulated	0	0	0	0	0	0	0	0	0

The repeated centrifuging had no effect because 95 per cent sporulation resulted in the control tubes.

No development took place when the cultures were incubated in 4 per cent formaldehyde, or 2 per cent Liquor Cresolis Compositus, but 95 per cent of the oöcysts developed in 2 per cent copper sulphate.

5. Effects of Sunlight and Drying

Freshly collected cultures of bovine coccidia were poured in open Petri dishes and exposed to the direct rays of the sun for a total of 24 hours. No preservative was added to two of the dishes, while the other two contained 1.5 per cent potassium dichromate. Preservative was added to the cultures and they were placed in test tubes and aerated daily for three weeks, but no development took place. The same results were obtained from a later trial.

Two Petri dishes of culture, one of which contained a preservative, were dried for 15 days at 20° C. Two other identical samples were dried for 15 days out of doors, exposed to sunlight and a mild temperature. Water was added to dissolve the dried material. Only oöcyst shells were found upon the examination of centrifuged samples. A portion of culture E was added to sterilized soil and allowed to dry for 20 days. Examinations at intervals showed decreases in the numbers of oöcysts. Only oöcyst shells could be found at the end of the period.

A sub-culture was placed in a 12 x 20 inch flat bottomed porcelain pan. The depth of the culture was about one-eighth of an inch. This was dried at 20° C for 48 hours and examined. Nothing but oöcyst shells remained. This test was repeated with similar results.

Fecal material, of a solid consistency, containing large numbers of oöcysts, was allowed to dry for 15 days at room temperature. No oöcysts could be found after this period of drying.

6. Effects of Putrefaction

Freshly collected samples of bovine coccidia containing a large amount of hemmorhage were placed in the incubator and paraffin oven. The temperature of the incubator was 37° C, while the temperature in the paraffin oven was about 58° C. The cultures were removed after a month's exposure to these temperatures and 1.5% potassium dichromate was added. They were aerated daily for a period of three weeks, and then examined. No sporulation had taken place; the oöcysts still retained their shape, but they showed a marked contraction of the cytoplasm.

Fresh material was placed at -5° C for one month. Preservative was added and the culture was aerated daily for two weeks. Ninety-five per cent of the oöcysts segmented. The hemorrhage in this sample caused no destruction of the oöcysts at this low temperature.

A pure culture (D) of E. zurnii containing almost pure hemorrhage stood at room temperature for five days. Preservative was added and the culture was aerated daily for a week. Complete sporulation resulted and there was apparently no marked destruction of oöcysts.

Screened cultures of oöcysts in which no visable hemorrhage was present have stood at room temperature, with no preservative, without marked destruction of oöcysts. The viability was shown by sporulation trials. A large amount of the same culture was stored at 22° C for two months. Rapid sporulation of the oöcysts took place when 2 per cent copper sulphate was added and the culture aerated.

Bovine coccidia are not as susceptible to putrefactive changes as is generally believed. Non-sporulated oöcysts are probably more resistant to these changes than the segmented forms. It would seem that putrefactive changes would be more severe if intestinal hemorrhage were present in the feces. Oöcysts present in manure piles are probably readily destroyed by the resulting decomposition and high temperature.

7. Artificial Digestion

Artificial digestion trials were made with twelve hooded rats confined in the laboratory. Two rats were killed. The intestines were stripped of fecal material, and centrifuge samples were run. No coccidial oöcysts were found. The remaining rats were starved for two days, then a very rich portion of culture E was fed in milk. Two rats were killed one hour later, four more in 24 hours, and the remaining four were killed 48 hours after feeding. Sporulated and non-sporulated oöcysts of bovine origin were found in the intestinal content of all the rats. One of the last ones to be killed showed a marked hemorrhagic enteritis in the small intestine. An examination showed a rather heavy infection of coccidia. A few sporulated and non-sporulated oöcysts of E. zurnii and E. smithi were found. It was not difficult to distinguish between bovine coccidia and the coccidia of the rat, because the latter were quite large, and although they were somewhat ovoid in shape, there was a distinct point at the small pole.

To further check this experiment, it was decided to repeat the trial. Seven half grown rats were placed in a sterile cage and fed sterilized grain and pasteurized milk. Composite fecal samples were negative for coccidia for two weeks. The rats were dosed three times *with culture E fed in milk with a pipette. Both sporulated and non-*sporulated oöcysts were passed in the feces. After the last feeding, two rats were killed in 1 hour, two in 24 hours, and two in 48 hours.

Fecal examination showed undigested oöcysts in all cases. The finding of segmented oöcysts of bovine coccidia in the intestine of a rat after 48 hours would indicate that no digestion of the oöcysts had resulted.

8. Sporulation Trials

a. Methods

Potassium dichromate is universally used as a preservative because it retards putrefaction and furnishes some oxygen to the cultures. Very little is known of the factors necessary for natural sporulation.

A number of attempts were made to sporulate bovine coccidia in the absence of a preservative, and to reduplicate, as much as possible, the required factors of natural sporulation.

Two quarts of sterilized dirt and culture E were thoroughly mixed, and placed in a 12 by 20 inch flat bottomed porcelain pan. The depth of the culture was three-fourths of an inch. This was stirred daily to aerate and just enough water was added to keep the dirt saturated. This experiment continued for five weeks, but only an occasional sporulated oöcyst could be found in repeated centrifuged samples. No odor of putrefaction in this culture could be detected.

The same procedure was followed in preparing another soil culture. This material was spread one-eighth inch deep in a box 2 by 3 feet. A wet towel was spread over the culture daily to supply moisture. Although the oöcysts could be found in great numbers in centrifuged samples, no sporulated ones were seen.

Fecal material, containing enormous numbers of coccidia, and an equal amount of sterilized soil, was placed in a half-gallon fruit jar. Water was added to make up for evaporation. The culture was aerated once or

twice daily by forcing air to the bottom of the solution with a pressure bulb. Only an occasional sporulated oöcyst could be found, but the material was later proved to be infective by feeding it to a susceptible calf. A small portion of this material was kept at 37° C for three weeks, but no development of the organisms resulted.

A pail of litter was collected from an infected stall in the college dairy barns and kept in the laboratory. Water was added regularly to keep the material moist. Only one segmented oöcyst was found, although repeated examinations were made. Sporulation was probably prevented by the continued decomposition that occurred.

No sporulation took place in cultures of bovine coccidia to which only water had been added. About 25 per cent of the oöcysts of an ovine culture sporulated in water before marked decomposition had taken place.

Two per cent copper sulphate was tried as a preservative for cultures and compared with 1.5 per cent potassium dichromate. No difference could be noticed in the rate or percentage of sporulation.

b. Sporulation Time

The sporulation times for the different species of bovine coccidia reported by various investigators have varied. These variations are probably a result of failure to provide optimum conditions for sporulation. Tyzzer (33) had pointed out that rapid and maximum sporulation of chicken coccidia can be attained by placing small amounts of the potassium dichromate culture in open Petri dishes exposed at room temperature. This is

unsatisfactory to sporulate bovine coccidia when there is a large amount of fecal material. The sporulation times given by Wilson (58) are as follows: E. cylindrica 2 to 10 days, E. zurnii 12 to 20 days, and E. smithi 14 to 21 days.

Bovine coccidia when aerated in large containers do not become infective in a short period. Three quart fruit jars, each containing 200 cc of a sub-culture of culture B, were placed at 37° C and aerated four or five times daily. This culture contained oöcysts of E. cylindrica, E. zurnii and E. smithi. The oöcysts were freed from the potassium dichromate by washing and centrifuging the material four times before the calves were dosed.

Calf No. 10 was drenched with 200 cc of sub-culture B, which had been incubated 24 hours. Fecal examinations were made for a month. No infection resulted.

Calf No. 11 was given 200 cc of sub-culture B, which had been incubated 72 hours. No infection was produced. This calf was later proved to be susceptible by feeding 150 cc of culture A.

Calf No. 12 received 200 cc of sub-culture B, which had been 120 hours in the incubator. Fecal examinations remained negative, for Eimeria oöcysts.

The possibilities for rapid sporulation were suggested when centrifuged samples, which had stood for 12 hours, were examined and sporoblast formation was noted in oöcysts of E. cylindrica and E. zurnii. There was no apparent development in E. smithi. About 200 cc of a sub-culture of culture E, containing about 90 per cent E. zurnii, 7 per cent E. cylindrica, and 3 per

cent E. smithi, was screened and placed in a 6 by 8 inch flat bottomed pan. Potassium dichromate and a small amount of concentrated sugar solution were added. Sixty per cent of E. cylindrica sporulated after 24 hours incubation, and appeared to be fully sporulated when examined after 48 hours. Sporoblast formation had taken place in E. zurnii in 24 hours and after 48 hours 35 per cent were fully segmented. Complete development had occurred at 72 hours. Sporoblast formation was noted in an occasional oöcyst of E. smithi after 72 hours incubation. An examination after 96 hours showed that plasmolysis had caused a marked destruction of E. zurnii and a few oöcysts of E. cylindrica had degenerated. About 1 per cent of E. smithi was sporulated. This material was dialyzed.

Calf No. 13 was drenched with this culture. On the twelfth to the seventeenth day E. zurnii were passed in small numbers. Large numbers of E. cylindrica were passed in pure culture from the twenty-third to the twenty-eighth day. On the day following E. cylindrica and E. smithi were found. Oöcysts were passed in great numbers for a short time. A diarrhea developed on the twenty-first day and on the twenty-sixth day the feces were streaked with hemorrhage. No severe symptoms of the disease were shown.

Freshly collected feces, containing large numbers of E. cylindrica was screened and potassium dichromate was added. This culture was added to a flat bottomed pan to a depth of a quarter of an inch. Complete sporulation had occurred in 95 per cent of the oöcysts in 48 hours. The same results occurred in two succeeding trials with the same organisms.

Pure cultures of E. smithi were treated in the same manner, and sporulation occurred in 3 to 5 days.

The sporulation time is given for the three species of Eimeria in cattle:

Eimeria cylindrica - 24 to 48 hours

Eimeria zurnii - 48 to 72 hours

Eimeria smithi - 3 to 5 days

To test the infectiveness of E. cylindrica after 48 hours incubation, a pure culture of the organism was fed to a susceptible calf, No. 14. Elimination of Eimeria cylindrica in pure form began on the tenth day (May 24, 1932.)

c. Observation of Morphologic and Physiologic Characteristics of the Genus Eimeria Affecting Cattle.

Table 2. Comparisons

Characteristics	Eimeria zurnii	Eimeria cylindrica	Eimeria smithi
Sporulation time	48 to 72 hours	24 to 48 hours	3 to 5 days
Major axis	18 u	23.7 u	28 u
Minor axis	17.3 u	14.4 u	20.5 u
Shape of oöcyst	Spherical	Cylindrical	Egg shaped
Oöcyst's wall	Uniform-colorless	Uniform-colorless	Thin at small pole Colorless
Residual Body	None	In spore	In spore
Shape of sporocysts	Ovoid	Cylindrical	Elongated

The following data were computed from the measurements of 50 consecutive oöcysts as they appeared on a slide from pure cultures of two species of coccidia:

Eimeria cylindrica*

Major axis		Minor axis	
s d	= ± 1.23	s d	= ± .2
rs	= ± .9	rs	= ± .135
rm	= ± .2	rm	= ± .04

Eimeria smithi

Major axis		Minor axis	
s d	= ± 1.06	s d	= ± .279
rs	= ± .74	rs	= ± .6
rm	= ± .23	rm	= ± .2

Experimentation with pure cultures of bovine coccidia have shown that the morphologic and physiologic features of the subcultures are identical to those of the parent cultures. With careful consideration of the evidence, it becomes apparent that Eimeria zurnii, Eimeria cylindrica, and Eimeria smithi, are three separate and distinct species of bovine coccidia.

Examination of culture E has shown the presence of very large oöcysts which measure about 46.2 u by 25.2 u. The oöcysts are so large that the elongated sporocysts float free inside. The sporocysts have a large residual

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*S. D. = Standard Deviation - R.S. Error, single observation

R. M = Mean

body. The oöcyst wall is stained a dark green color. Many investigators have stated that the oöcyst wall of E. smithi may be either colorless or stained. Only one of the many cultures used during this study contained these large stained oöcysts, and when this culture was fed to calves, there were considerable numbers passed during the course of the infection. No stained oöcysts were found in any of the several pure cultures of E. smithi. The oöcysts are egg shaped, and they sporulate in about five days. From a careful comparison of these large oöcysts with those of E. smithi, it is believed that this large form of Eimeria is a distinct and unnamed species of bovine coccidia.

F. SYMPTOMS

The symptoms of bovine coccidiosis have been well described in the literature. The severity of the symptoms shown in coccidiosis vary markedly. The predominant symptom is a diarrhea which is usually mild at the onset of the disease. This may rapidly change to a severe diarrhea with marked hemorrhage, and large amounts of mucus and long shreds of epithelium are passed with the feces. A nephritis, which is manifested by the passage of bloody urine, may accompany the diarrhea. If the animal's resistance is very low, secondary intestinal infection, or pneumonia, may result. Emaciation is rapid. The animal will eat, but the appetite is poor. The eyes are sunken, the hair coat rough, the pulse is accelerated, and a general anemic condition is apparent. The animal becomes dull and listless, will stand with the back arched and the abdomen drawn up. Severe intestinal pain is further evidenced by a grinding of the teeth and a drooling of saliva. Calves recuperate very slowly from severe infections.

G. PATHOLOGY

The gross pathologic changes in bovine coccidiosis are confined to the rectum, colon, and caecum. They are, loss of surface epithelium, hemorrhage, and a thickened and cooked appearance of the mucosa. Large areas may be entirely denuded of the epithelium. The hemorrhages vary from petechial to diffuse hemorrhages. These lesions are usually noticed on the crests of the ridges. The three species of bovine coccidia invade the deeper portions of epithelium in the intestinal glands, where all developmental stages may be found. The rectum usually shows more involvement than the other portions of the intestine, although occasionally the lesions will be more numerous in the caecum. Catarrhal enteritis may be present in the small intestine, but microscopic lesions are rarely found there, except at the terminal portion of the illeum. No marked differences in the developmental stages of three species could be found, but the sexual forms of E. smithi are much larger than the other two.

It is highly important that a careful study of the pathology of bovine coccidiosis should be made.

IV. DISCUSSION

In experimental work with bovine coccidiosis, it is important to secure susceptible, non immune calves. All possible sanitary precautions must be taken to prevent accidental infection. The most rigidly controlled conditions seem to be necessary in the study of the disease in lambs. Accidental infection does not occur in cattle as easily as in other animals; this is probably due to a difference in the physiologic characteristic of the oöcysts. It is important to use pure cultures of the different species to avoid confusing results.

Results of experimental and field cases of coccidiosis indicate that natural infection occurs through the ingestion of small numbers of ripened oöcysts, possibly over a long period. The infection is probably spread in a herd by immune animals acting as carriers of the organisms. It seems quite evident that animals have a natural resistance and that a protective immunity against subsequent infections may result from a severe attack of coccidiosis. No fatalities occurred among the nineteen calves artificially infected, and there were no correlations between the size of the doses given and the resulting infections. This suggests some predisposing factor which influences the course of the infection. There is slight evidence to show that oöcysts which are sporulated without a preservative produce a more severe infection than those sporulated in 1.5 per cent potassium dichromate. A study of the courses of the infections indicates that the resistance of the host has direct influence on sexual or asexual development of the parasite.

The results of the cross infection experiments were confusing. Lambs which were fed bovine coccidia developed an infection, but accidental infection occurred in one of the controls. This shows the necessity of controlled sanitary conditions. A culture collected from these lambs produced an infection when fed to a susceptible calf. Since no cases of accidental infection had been noted in the experimental calves, it was concluded that the calf was either susceptible to the oöcysts of ovine origin, or that bovine coccidia were present in the culture fed. The oöcysts fed by the lambs did not conform to any of the descriptions given for the Eimeria parasitic in sheep. There was a certain similarity between the oöcysts passed by the lambs and those passed by the calf. It is highly important to know whether cattle will contract the infection from sheep. Further experimentation is necessary in order to establish this questionable possibility of cross infection.

Both sporulated and non-sporulated oöcysts have a low thermal death point and are destroyed by sunlight, drying, and putrefaction. The oöcysts are very resistant to freezing temperatures. The number of oöcysts in a culture decreases with age. Non-sporulated oöcysts may retain their ability to sporulate and their infective power for at least a year and a half.

Artificial digestion trials have shown that rats will not digest bovine coccidia.

A large percentage of bovine coccidia will not sporulate unless some preservative is added to the culture. Sporulation trials have shown that great numbers of the oöcysts of three species of coccidia will sporulate and become infective in a very short time under favorable conditions. The different morphologic and physiologic characteristics of three species of coccidia have been noted. Pure cultures of these species retain their characteristics after repeated infections. It is concluded that E. zurnii, E. smithi, and E. cylindrica are valid species.

V. CONCLUSIONS

1. Much of the past confusion concerning bovine coccidiosis has been eliminated by recent experimentation.
2. Natural infection probably results from a continuous ingestion of small numbers of ripened oöcysts, with immune carriers spreading the organism.
3. The natural resistance of calves may vary. An acquired immunity, which protects the animal against subsequent infections for at least five months, may result from a severe coccidial infection. Old animals probably have, as a rule, a well developed immunity.
4. In this study there was found to be no correlation between the amount of infective material fed to a susceptible calf and the resulting infection.
5. Failure to produce severe artifical infections has suggested the possibility of some predisposing factor. It is believed that sporulation of the oöcysts in potassium dichromate may attenuate them to some extent.
6. There does not seem to be a definite cylical character of development with a limited number of asexual generations in bovine coccidiosis. Evidence indicates that the course of the infection is influenced by the resistance, or immunity, of the host determining whether the development of the parasite will be either sexual or asexual.
7. Although cross infection experiments were not entirely satisfactory or conclusive, yet evidence at hand indicates that lambs may be susceptible

to the Eimeria of cattle, and vice versa. Further work should be done on this phase of the subject.

8. From this study it would appear that lambs are highly susceptible to infection with Eimeria. From the pathologic study of one case of the infection, it would appear that the organisms developed primarily in the caecum and involve the superficial layers of the epithelium. This is further borne out by the clinical symptoms observed in nine lambs.
9. The characteristics of the oöcysts passed by these lambs used in this study did not conform morphologically to any of the Eimeria described for sheep.
10. Non-sporulated oöcysts will probably survive the winter temperature encountered in Virginia.
11. Non-sporulated oöcysts seem to retain their viability at refrigeration temperature longer than sporulated ones.
12. Both non-sporulated and sporulated oöcysts are readily destroyed by the action of sunlight, drying, and putrefaction.
13. Sporulated oöcysts from cattle fed to young rats were not digested.
14. We were unable to sporulate any great numbers of bovine coccidia unless a preservative was used. Copper sulphate seems to be as efficacious as potassium dichromate as a preservative for sporulating bovine coccidia.
15. Sporulation time for some of the species of bovine coccidia is not as long as commonly believed.
16. The morphologic characteristics of one type of oöcyst encountered appeared not to conform to any of the described bovine species.

VI. DESCRIPTION OF PLATES

Plate I.

Figure 1 is a photomicrograph of schizonts showing daughter nuclei, in the illeo-caecal valve of a calf four days after feeding the infective material. X 750.

Plate II.

Figure 2. Sexual forms of Eimeria smithi. Illeo-caecal valve. X750.

Plate III.

Figure 3. Stages of development of the organism in caecum of a lamb. X350.

Plate IV.

Figure 4. The same as figure 3. X750.

Plate V.

Figure 5. Eimeria smithi in pure culture showing sporulation after 4 days incubation. X750.

Plate VI.

Figure 6. Eimeria cylindrica in pure culture after 43 hours incubation. X750.

Plate VII.

Figure 7. Oöcysts of Eimeria cylindrica and Eimeria smithi after 43 hours incubation. X750.

Plate VIII.

Figure 8. An oöcyst which measures 46.2 u by 25. 2 u. Photomicrograph shows one sporocyst on end. The two sporozoites and a definite residual

body can be seen. X750.

Plate IX.

Figure 9. Photomicrograph shows two size ranges of oöcysts from an infected lamb. X750.

Plate I.

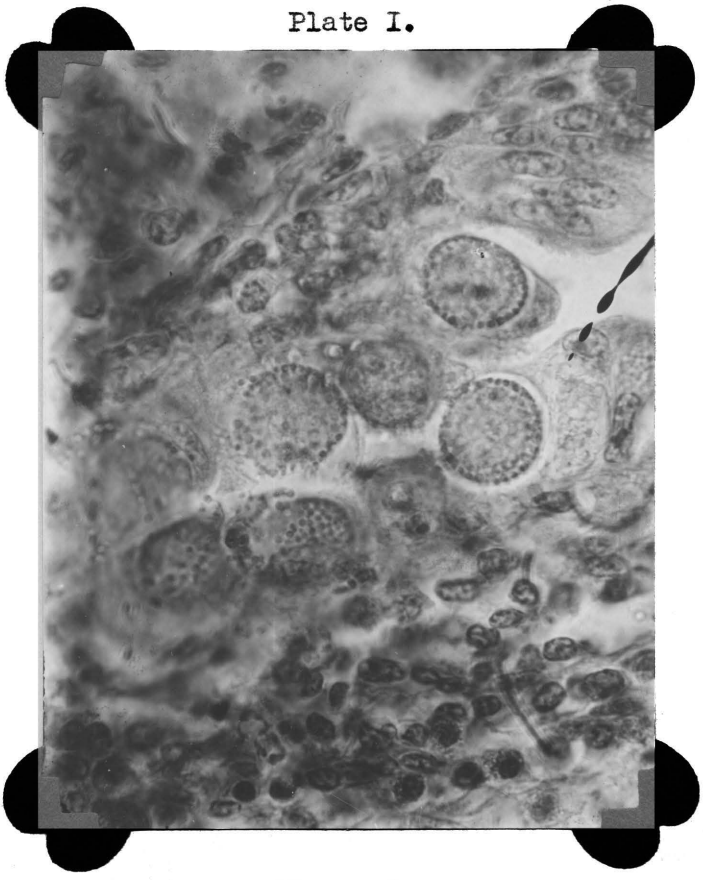

Figure 1.

Plate II.

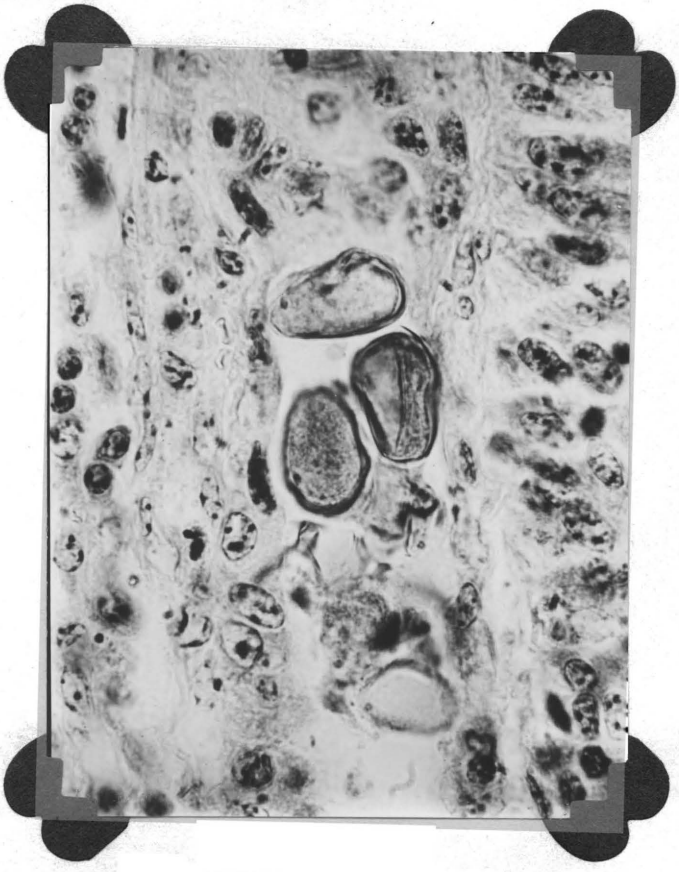

Figure 2.

Plate III.

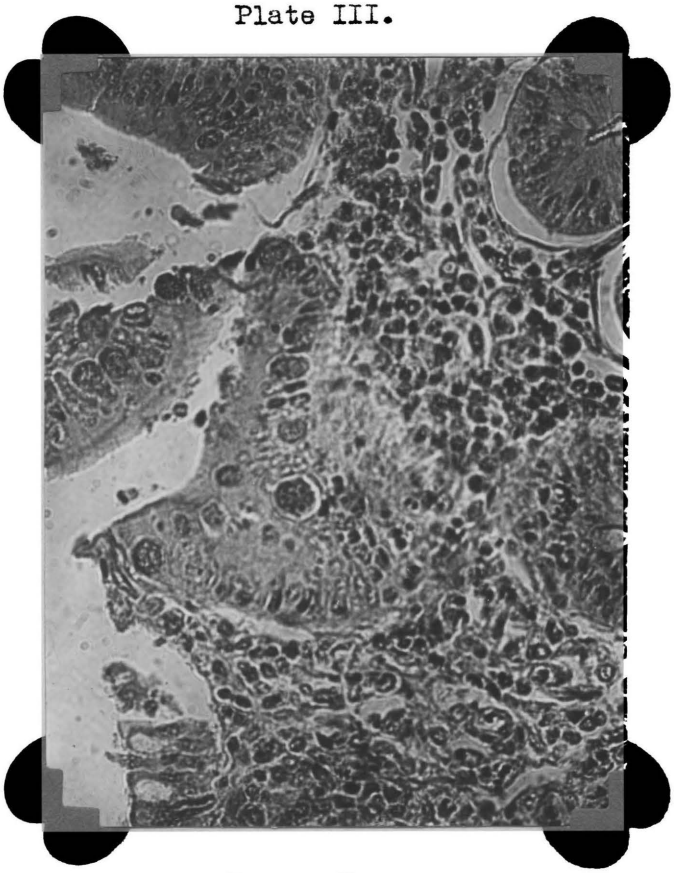

Figure 3.

Plate IV.

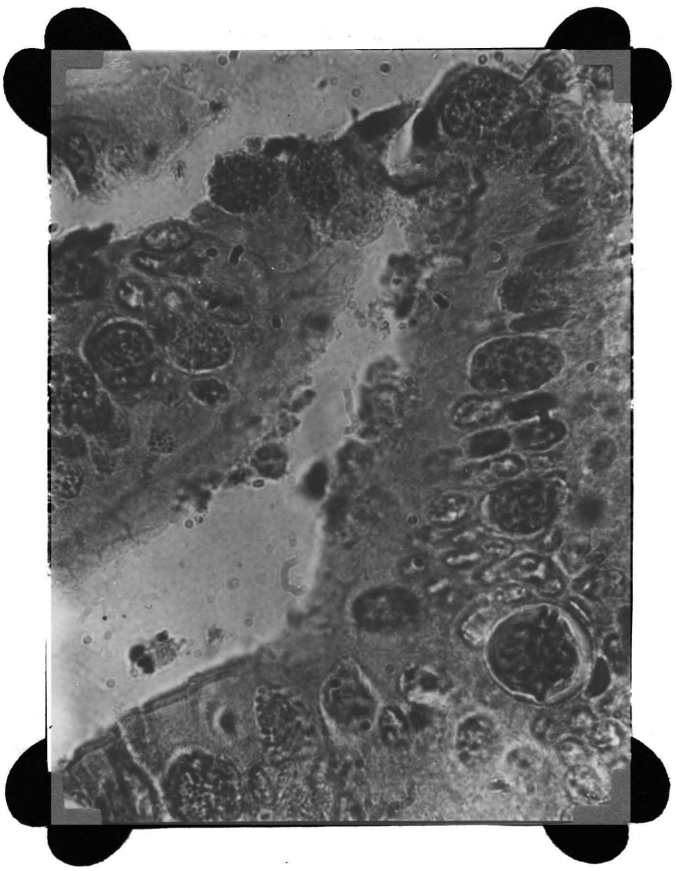

Figure 4.

Plate V.

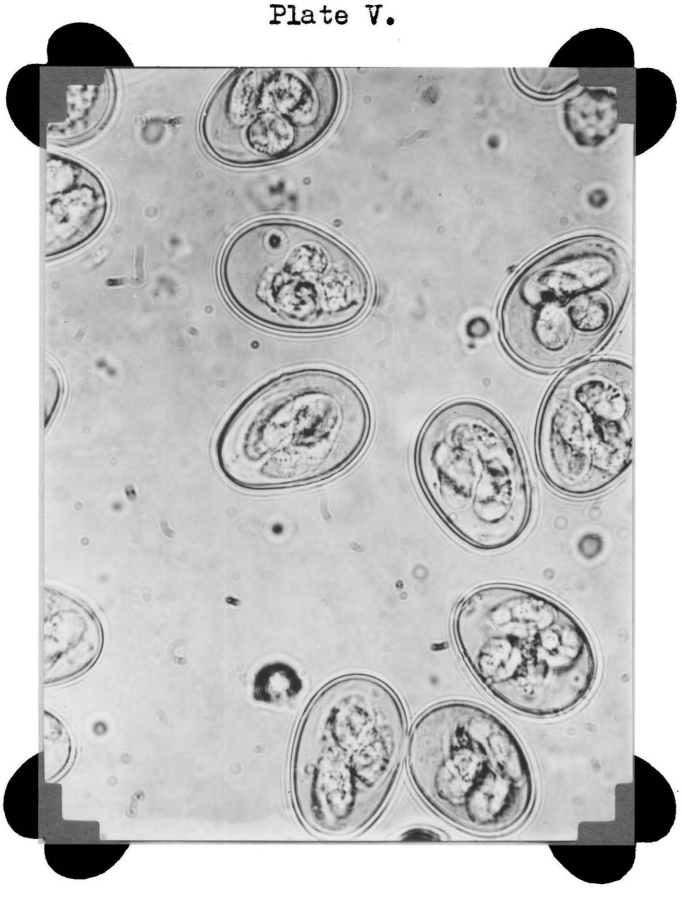

Figure 5.

Plate VI.

Figure 6.

Plate VII.

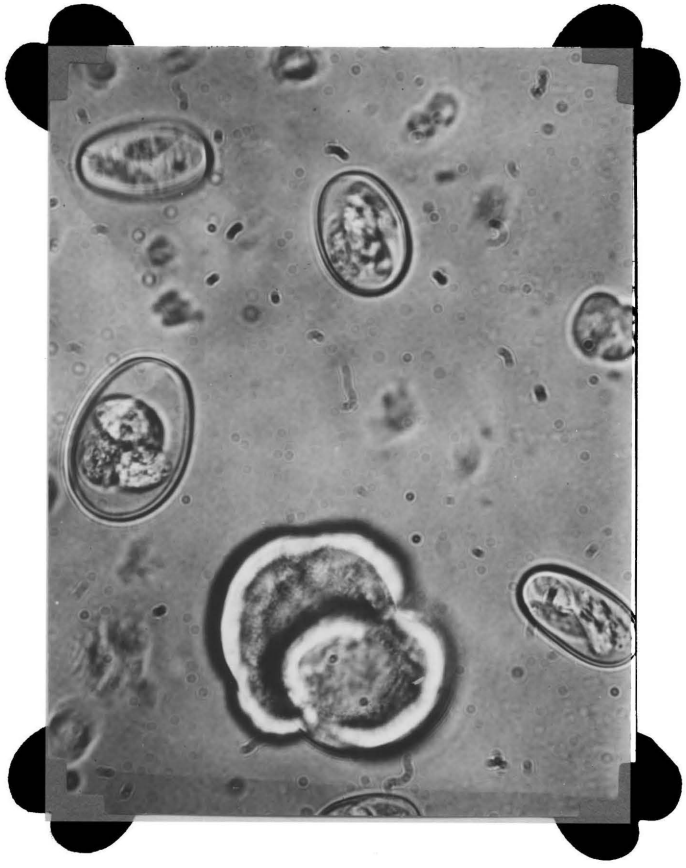

Figure 7.

Plate VIII.

Figure 8.

Plate IX.

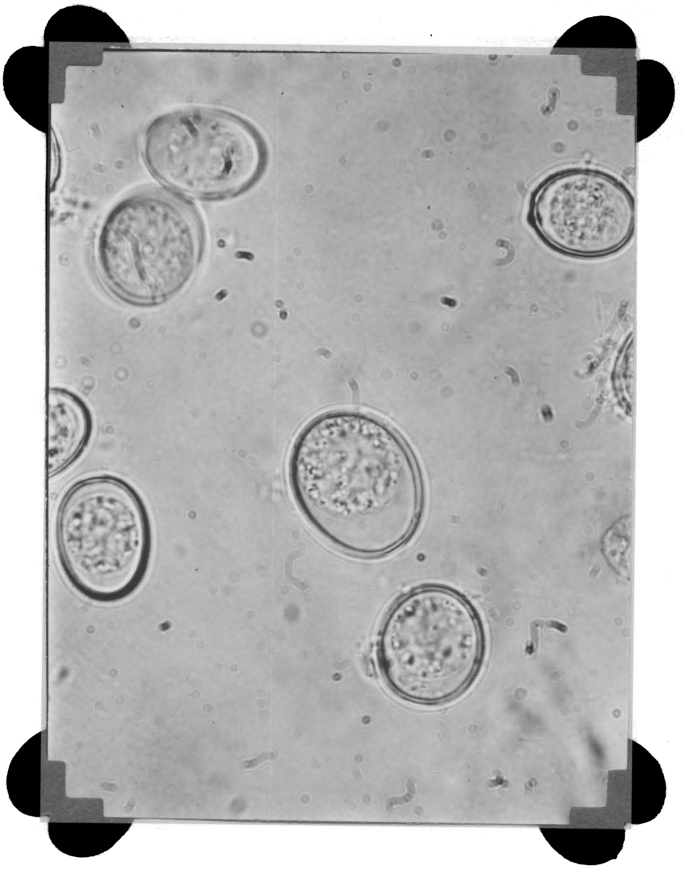

Figure 9.

VII. ACKNOWLEDGEMENTS

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