

EXPERIMENTALLY INDUCED PORCINE COCCIDIOSIS

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SUMMARY

Two controlled field experiments in which the performance of groups of pigs were studied following daily exposures to large numbers of mixed sporulated coccidial oocysts are reported. Comparisons between control and principal groups were made on the basis of faecal oocyst counts (OPG) and body weight gains.

On the basis of oocyst counts, experimentally infected principals in both experiments developed substantial oocyst populations which reached a peak on the 9th day of infection and declined steadily thereafter. Environmental controls did not develop oocysts. Controls gained more weight on the average than the infected principals during the experiments. Microscopic examination of intestinal tissue sections from an infected principal that died on day 22 revealed the presence of several large cystic bodies in the mucosa of the caecum and large colon. It is suggested that these structures could be schizonts.

INTRODUCTION

The Swine Coccidians first came to prominence in 1878 when Rivolta and Zurn, both working independently, encountered oocysts in the faeces of pigs. Since these pioneering efforts, numerous reports have recorded the widespread nature of the swine coccidians, and the types of species parasitizing swine. Unlike its counterparts in other domestic animals however, the swine coccidians are more or less unrecognised as potential disease causing organisms.

It is probable that all coccidial are potentially capable of inflicting damage on their hosts. Indeed, with certain species of Bovine, Avian and Ovine coccidians, pathological changes in the host have clearly been associated with the presence of the parasite. With the possible exception of Vetterling (1966) determined attempts to evaluate the pathogenicity of swine coccidia have not been made.

The present report describes 2 controlled field experiments in which groups of

pigs were studied to determine the clinical impact of daily administration of infective coccidial oocysts.

MATERIALS AND METHODS

Two studies with pigs were conducted over a 2 year period beginning in January, 1973. The weaned pigs were purchased periodically from Wisconsin dealer. At the time of purchase, each pig appeared to be healthy and seemed suitable for study and in fact were susceptible to infection by coccidians as was evidenced by the absence of oocysts in their manure prior to exposure (Tables 1 and 2). Daily faecal examinations of these pigs prior to and during the experiment revealed no evidence of worm infections.

In both experiments, pigs were weight balanced into exposed and non-exposed groups. Each pig was held in a separate pen and fed a ration consisting predominantly of grain supplemented with protein and trace mineral salts. Each pig received equal amounts of the feed daily.

Experimentally infected groups of pigs were exposed to the same number of sporulated oocysts in each experiment; the infective cultures contained a mix of species which were recorded and are depicted in plate 1, figure 2. The oocysts in aqueous suspension were introduced orally with a steel drenching syringe.

The parameters employed to monitor severity of infection were daily oocyst counts per gram of faeces (OPG), weekly body weight gains, and faecal consistency. Oocyst counts were made by subjecting 1gm faecal samples to centrifuged (spun) against glass coverslips and examined microscopically.

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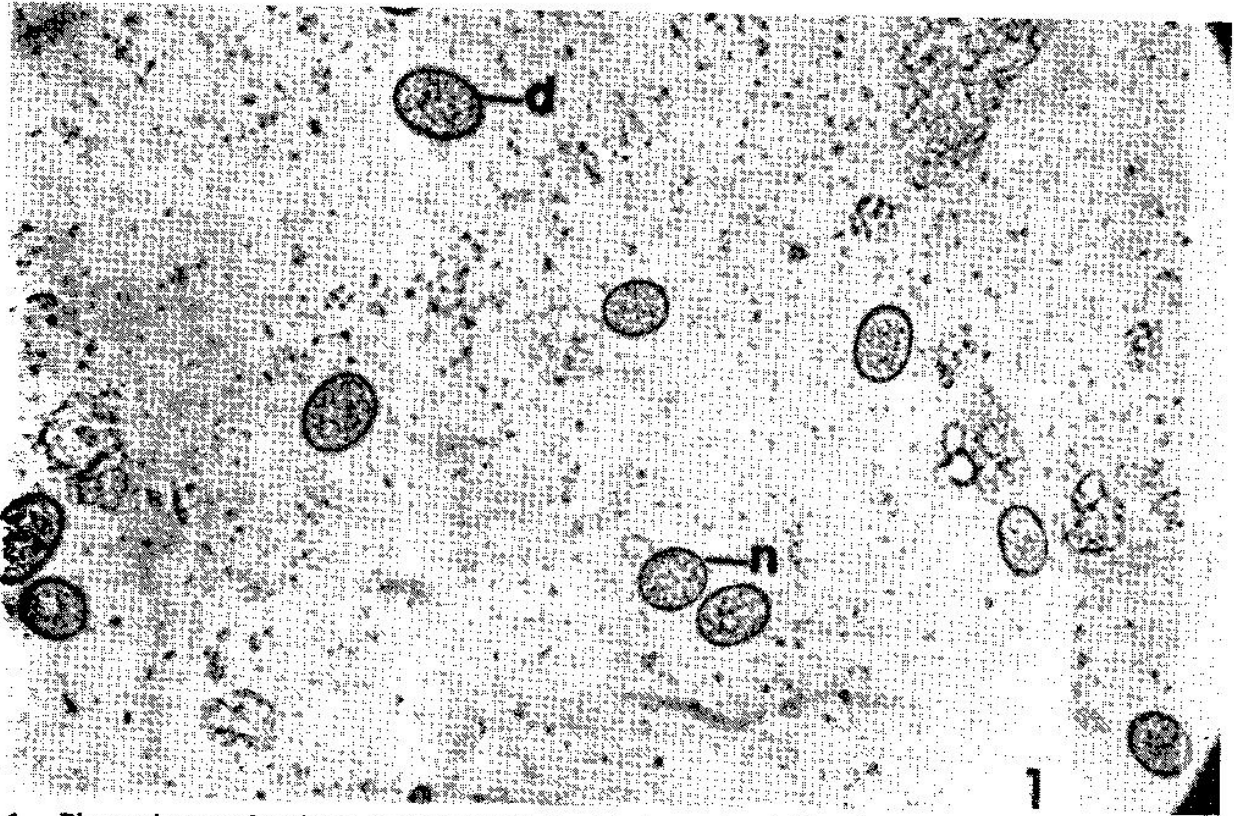


Plate 1. Photomicrographs of unsporulated and sporulated oocysts of *Eimeria* species of swine. Oocysts spun in sugar solution (specific gravity 1.27) against a glass coverslip and examined under a light microscope at 675X.

Fig. 1. Unsporulated oocysts of *Eimeria debliecki* Douwes, 1921 (d) and *Eimeria neodebliecki* Vetterling, 1965

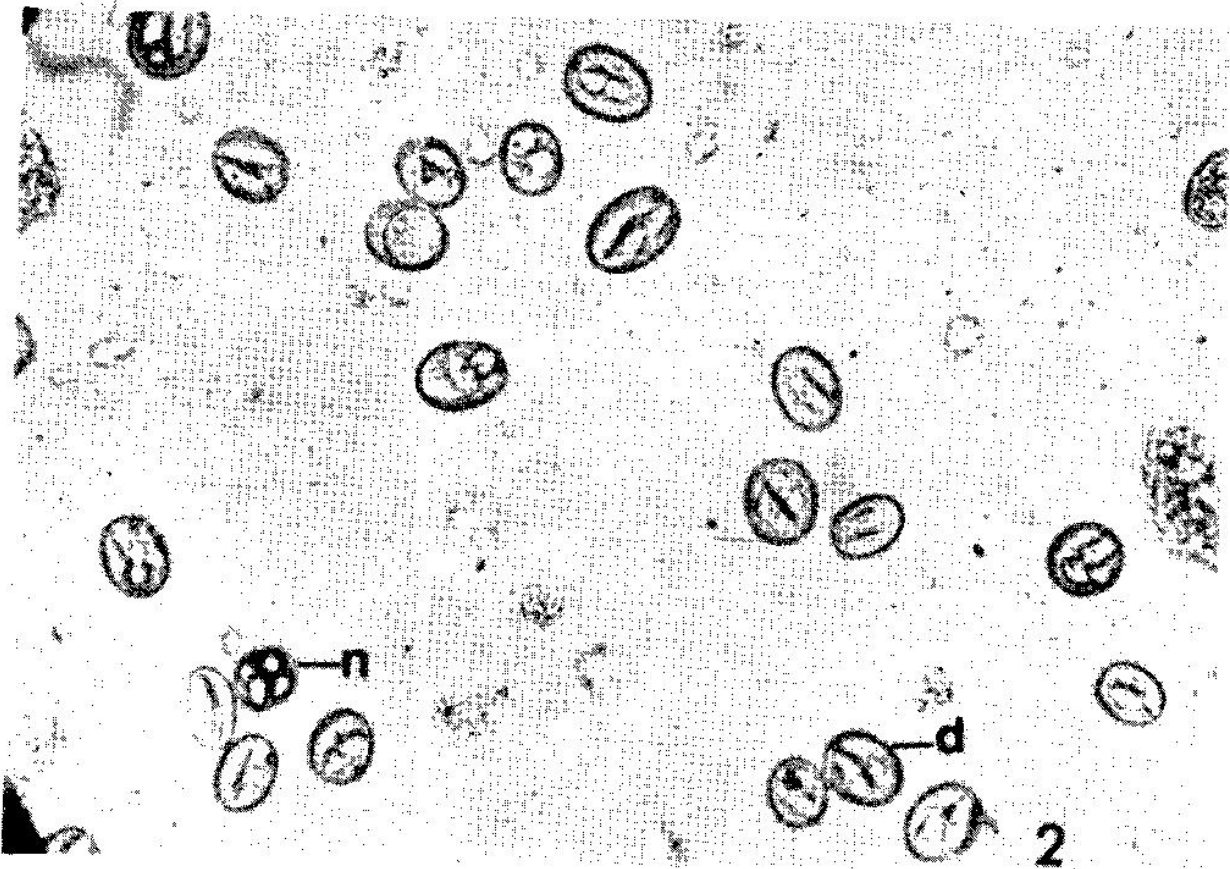


Fig. 2. Sporulated oocysts of *Eimeria debliecki* (d) and *Eimeria neodebliecki* (N).

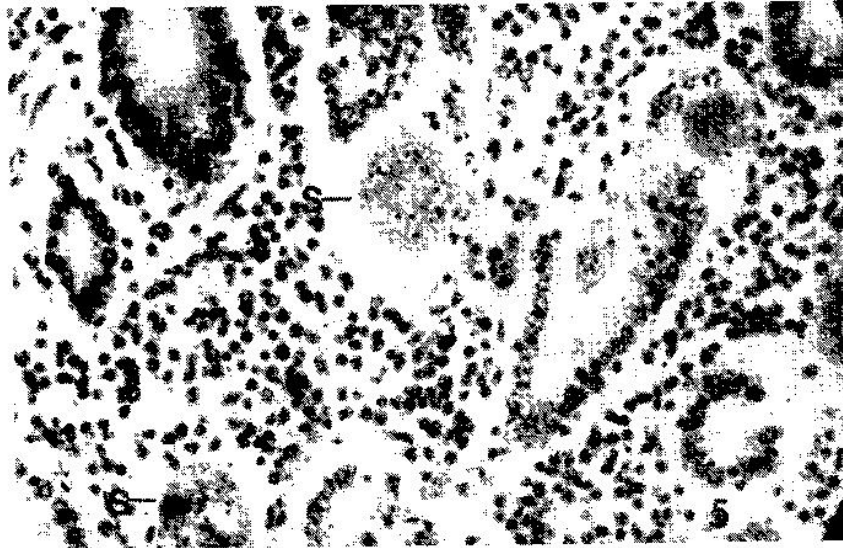


Plate 2. Photomicrograph of endogenous stages of *Elmeria* species of swine. Tissue section taken from the caecum and stained in Erlich's haematoxylin and eosin. Section observed under light microscope at 675x.

Fig. 3. Two shizonts (s) showing numerous randomly distributed nuclei. Both show compartment formation around the nuclei.

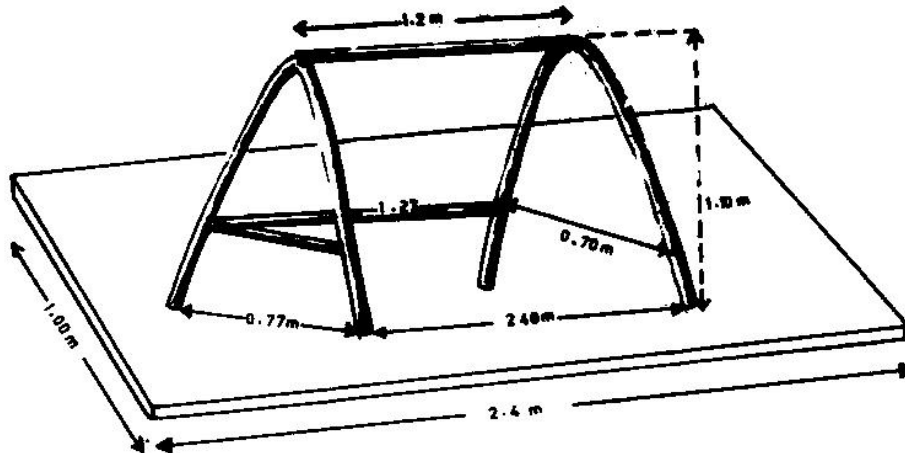


Fig. 1. A Model Artificial Dummy Sow.



Fig. 2. Collection of semen with an artificial vagina and a dummy sow.

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Plate 3. Photomicrographs of endogenous stages of *Eimeria* species of swine. Tissue Sections taken from the large intestine and stained in Erlich's haematoxylin and eosin. Sections observed under light microscope at 675x.

Fig. 4. Two schizonts (s) with several randomly arranged nuclei. Both show compartment formation around the nuclei.

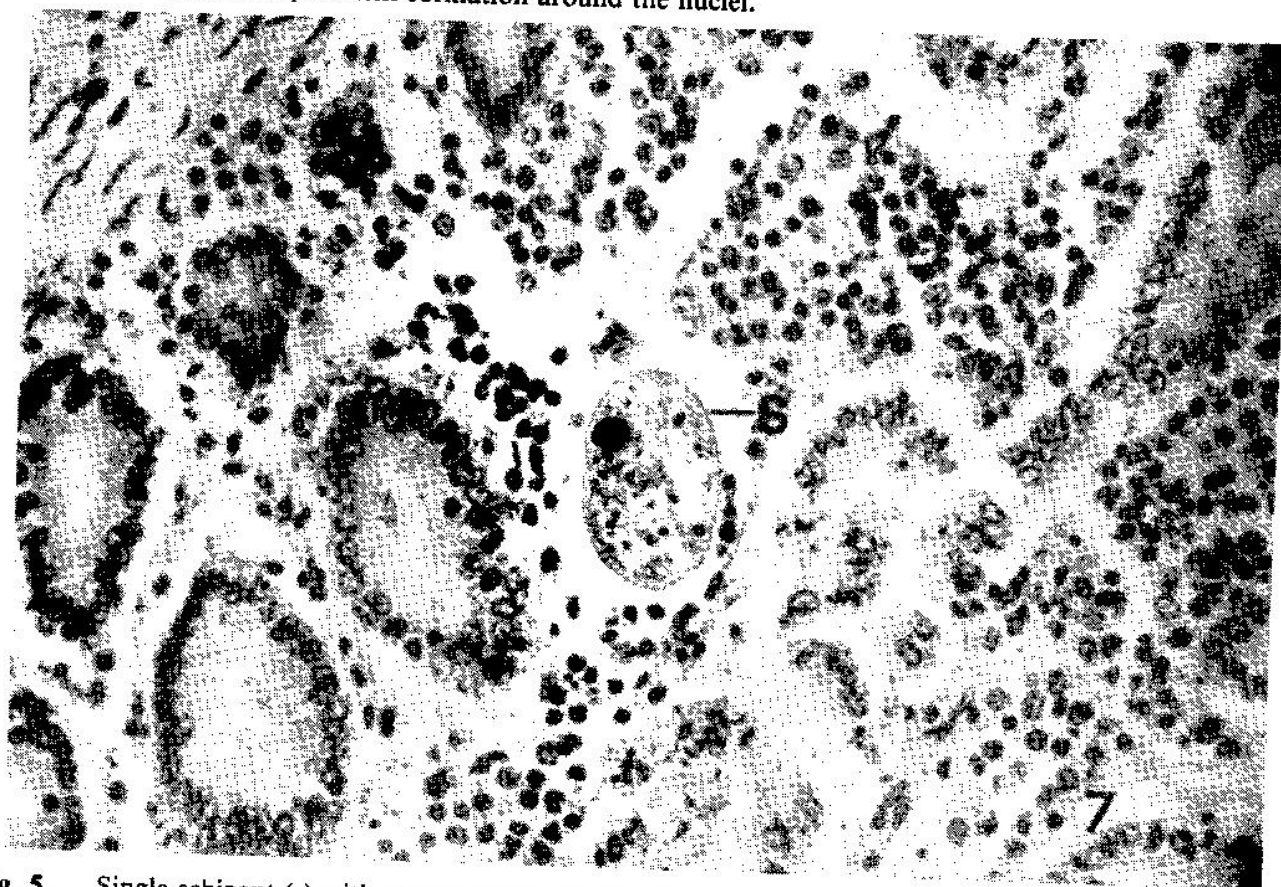


Fig. 5. Single schizont (s) with several randomly distributed nuclei. Appears vacuolated with compartment formation around nuclei.

Experiment 1:- The 1st Experiment involved 12 pigs weighing 7.7 to 10.5kg. They were assigned to 2 groups. Group 1 consisted of 8 pigs each of which was exposed to 100,000 sporulated oocysts in aqueous suspension, daily. The number of infective oocysts given each pig daily was increased to a maximum of 500,000 by day 6 and on day 23 exposure ceased. The infective culture was 77% sporulated and consisted of 66% *Eimeria deblickei*, 5% *Eimeria suis* and 3% *Eimeria perminuta*.

Group II was the environmental control. It consisted of 4 pigs which were not exposed to infective oocysts but rather received water placebos daily. This group was used to determine that only exposed pigs developed oocyst populations.

Experiment 2:- Fifteen pigs were utilized in the 2nd experiment; their initial weights ranged from 3.9 to 11.8kg. They were weight balanced into 3 groups. The first group (group III) consisted of 6 pigs each exposed to 300,000 sporulated oocysts in aqueous suspension daily, from day 0 through day 15. Exposure ceased on day 15. The infective culture was 78% sporulated and consisted of 76% *Eimeria deblickei* and 24% *Eimeria neodeblickei*. Seven days before infection, a sample of the infective culture was sent to the Wisconsin Animal Health Laboratory for bacteriologic examination. The samples were found negative on culture for salmonella and hog cholera virus but positive for mixed coliforms. The laboratory culture was subsequently treated with streptomycin sulphate, to which the coliform bacilli are susceptible, prior to exposure of experimental animals.

The second group (Group IV) was the environmental control. There were 5 pigs in this group. They were not infected but received water placebos daily. This group was used to determine that only exposed pigs developed oocyst showers.

A third group consisting of 4 pigs, was exposed to 300,000 sporulated oocysts daily. These were killed on infection days

2, 3, 4 and 5 respectively. After killing, the gastro-intestinal tracts were removed and cut at intervals of 31cm, slit open longitudinally and lightly rinsed in saline. Tissues were fixed in a 10% solution of formalin, embedded in paraffin wax and sectioned at 7u. Sections were stained in Erlich's haematoxylin and eosin and examined microscopically for endogenous stages of coccidia at 675X.

RESULTS

Experiment 1:- Oocyst counts illustrating the difference in coccidial populations which developed in the principals and controls are shown (Table 1) as group averages on selected days. In comparison with the environmental controls (Group II), the infected principals (Group I) developed substantial coccidial populations and produced showers of oocysts. For example, oocyst production by infected principals began on day 6 (310 o.p.g) and at peak discharge on day 9 averaged 6,060 o.p.g. Thereafter, oocyst production steadily declined even though fairly high levels were maintained through day 13 (1,565 o.p.g.). Oocyst elimination in the infected principals was associated with moderate, but intermittent, non-haemorrhagic diarrhea. Environmental controls had no diarrhea.

The data on weekly body weight changes show an apparent advantage by environmental controls over the infected principals (Table 1). At the end of the experiment, the infected principals lost an average of 0.5kg, whereas, the environmental controls averaged 3.6kg gain.

Experiment 2:- Infected principals (Group III) discharged oocysts as from day 6; peak production occurred on day 9 when they averaged 8,970 o.p.g. (Table 2). Oocyst production dropped considerably as from day 10 until day 27 when no oocysts appeared in the faeces. In comparison, environmental controls (Group IV) did not pass oocysts throughout the experiment. The data on body weight changes (Table 2) show an apparent ad-

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TABLE 1

Development of Coccidial Populations and Changes in Body Weights for Groups of Pigs Exposed to 100,000 to 500,000 Infective Oocysts Daily and Non-Exposed Environmental Controls

Day After Exposure	Faecal oocyst counts (per gm) by Day After Exposure		Body weight (kg) by days Before and After Exposure		
	Group I	Group II	Exposure Day	Group I	Group II
	8 Principals each exposed to 100,000 to 500,000 infective oocysts daily	4 Controls each receiving water placebo daily		8 Principals each exposed to 100,000 to 500,000 infective Oocysts daily	4 controls each receiving water placebo daily
6	310	0	-3	9.4	9.7
7	841	0	4	9.9	10.6
8	8549	0	11	9.3	11.3
9	6060	0	18	9.6	12.4
10	4190	0	25	8.9	13.3
11	3319	0	Group Gain	-0.5	3.6
12	1539	0			
13	1565	0			
14	788	0			
15	205	0			

TABLE 2.

Development of Coccidial Populations and Changes in Body Weights of a Group of Pigs Exposed to 3000,000 Infective Oocysts Daily and Non-Exposed Environmental Controls.

Days After Exposure	Faecal oocyst counts (per gm.) by Day After Exposure		Body weights (kg) by days Before and After Exposure		
	Group III	Group IV	Exposure Day	Group III	Group IV
	6 Principals each exposed to 300,000 infective oocysts daily	5 Controls each receiving water placebo daily		6 Principals each exposed to 300,000 infective oocysts daily	5 Controls each receiving water placebo daily
6	128	0	-3	6.4	6.5
7	350	0	4	7.2	7.4
8	1,240	0	11	7.8	9.1
9	8,970	0	18	8.1	10.3
10	5,115	0	25	*9.0	13.2
15	1,362	0	32	9.2	16.3
20	17	0	Group gain	2.8	9.8
24	*7	0			
27	0	0			

*One of the exposed principals died on day 22. Group averages for exposed principals are therefore based on 5 observations instead of 6 as from day 22.

vantage by the non-infected environmental controls (Group IV) over the infected principals (Group III). At the end of the experiment controls averaged 9.8kg. gain whereas the principals averaged only 2.8kg., an apparent advantage of 7kg.

From day 11, 4 of the 5 exposed principals (Group III) developed, non-haemorrhagic diarrhea; one pig in this group died on day 22 after scouring heavily for 11 days. A post-mortem examination of this pig revealed no gross abnormalities in the lungs, heart, liver and kidneys. The spleen however was shrunken and appeared blackish-red in colour. An examination of the gastro-intestinal tract revealed Oedema, necrosis and catarrhal enteritis which was most pronounced in the region of the caecum and large intestine as well as the posterior part of the ileum. The mesenteric lymph nodes however, did not appear enlarged. The gastro-intestinal tract of this pig was ligated at intervals of 31cm from rectum to stomach. Material removed from the lumen of each section was spun in sugar solution against a coverslip. Microscopic examination revealed oocysts in the jejunum and ileum and the number recovered increased with posterior progression i.e. more oocysts were recovered from the ileum than the jejunum. None was recovered from the duodenum and bile duct. Oocysts were recovered in large numbers from the caecum and colon. Oocysts recovered were not speciated and were therefore recorded as a mix of *Eimeria* species. After post-mortem examination, tissue sections from the gastro-intestinal tract were stained in Erlich's haematoxylin and eosin and examined microscopically for endogenous stages of coccidia. Several large cystic bodies were found embedded superficially or in the middle of the mucosa of the caecum and large colon-plates 2 and 3, figures 3—5. Cellular infiltration by plasma cells and lymphocytes was observed around these bodies. Eosinophils were also prominent in the same area but very few neutrophils were noticeable. A few of

these inflammatory cells appeared necrotic while a cellular exudate was observed surrounding or adjacent to these bodies. A total of 21 of these cystic bodies was counted from the caecum and large colon. They ranged in length from 30.8 to 61.6u (mean $46.2 \pm 8.5u$), while widths, ranged from 24.2 to 46.2u (mean $37.1 \pm 5.9u$). After careful morphological examination and comparison with stages illustrated in the literature, (Vetterling 1966) these cystic bodies were identified as schizonts. They did not however, contain distinct or well formed merozoites. Examination of tissue sections from the small intestine and other body organs did not reveal any endogenous stages.

Similar studies were conducted on a group of pigs exposed to oocysts on day 0 and killed 2, 3, 4 and 5 days post exposure respectively. The spleen, small intestine, heart, kidneys and liver of each animal appeared perfectly healthy upon visual examination. Stained sections of the gastro-intestinal tracts revealed no evidence of cell destruction or the presence of characteristic endogenous stages of the swine coccidians.

DISCUSSION

The pathogenicity of the swine coccidians has long been a bone of contention amongst parasitologists. In this study, the species of *Eimeria* used for infection were *E. deblickei*, *E. neodeblickei* and *E. perminuta*. Earlier studies have revealed necrotic enteritis, diarrhea and loss of body weight in pigs experimentally and naturally infected with *E. deblickei* (Boch and Wiesenhutter, 1963; Alicata and Willet, 1946. Vetterling /1966), on the other hand, observed no clinical effects in two week old pigs exposed to large inocula of *Eimeria deblickei* oocysts. The pathogenicity of *E. neodeblickei* (a relatively new species) and *E. perminuta* is unknown. In the present report, the performance of groups of pigs exposed daily to large numbers of infective oocysts was compared to non-exposed environmental

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controls. Exposure resulted in the development of large coccidial populations, measured by oocyst counts (Tables 1 and 2), in both principal groups. Peak discharge occurred on day 9 in both studies after which there was a gradual decrease in oocyst output — even though daily exposure to infective oocysts was continued in both studies. This phenomenon probably reflects a development of immunity to infection in exposed animals, the exact nature of which is unknown in swine coccidiosis. Biester and Schwarte (1932) and Rommel (1970), believe that immunity to swine coccidiosis is of very short duration lasting between 18 days to 4 months. It is possible on the other hand, that the decline in recoverable oocysts reflects a decrease in the number of epithelial cells available for parasitization due to the large numbers of oocysts fed each animal daily. This of course would lead to the expulsion of large numbers of liberated sporozoites, merozoites and gametes which failed to find cells to inhabit.

Weight gains of exposed principals in both experiments reflected the harmful effects of the infection upon production traits. Environmental controls in both experiments gained more weight than did the infected principals upon termination of the trials.

As has been the case with many investigations of swine coccidiosis, considerable difficulty has been encountered in these studies in locating and identifying endogenous stages of the parasite. Endogenous stages could not be recovered from tissues removed from 4 pigs killed 2, 3, 4 and 5 days after infection respectively. Several large cyst-like bodies later identified as schizonts were however found scattered in the mucosa of the caecum and large colon of a pig in group III which died on infection day 22, (Plates 2 and 3, Figures 3—5). Previous reports had established that the endogenous stages of the swine coccidians were located in the villi of the small intestine (Boch and Wiesenhutter, 1963; Vetterling,

1966; Rommel and Ipczynski, 1967). Surprisingly, none was found in the small intestine in the present study. The endogenous stages identified in the caecum and colon varied greatly in size ranging from 30.8 to 61.6u (mean $46.2 \pm 8.5u$) in length and 24.2 to 46.2u (mean $37.1 \pm 5.9u$) in width. The literature indicates that these bodies are unusually large for endogenous stages of coccidia (Vetterling, 1966; Rommel and Ipczynski, 1967). Consideration was given to the possibility that the cysts found could be nodules created by the presence of the larvae of the nodular worm (*Oesophagostomum*) or whipworm (*Trichuris* spp). Careful morphological examination however, revealed that this premise had no foundation since nodules created by these parasites are significantly larger than those found (Levine, 1968; Dunn, 1969) in the present study. In addition, the stages observed had a distinctly granular appearance and contained darkly stained bodies (nuclei) characteristic of the asexual stages of the swine coccidians. It is suggested, therefore, that these cysts could be immature schizonts.

These studies indicate that further work with the swine coccidians is essential. The data show that they develop readily in the host and exert some adverse effects on production traits. Studies are required to determine the precise locations of cellular infiltration and destruction working preferably with monospecific cultures.

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