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PRODUCTION OF BOVINE COCCIDIOSIS WITH *EIMERIA ZUERNII*

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Introduction

Bovine coccidiosis, mainly due to *Eimeria zuernii*, was estimated to cause an annual economic loss of approximately $4,000,000 in Canada (7). The disease has proved difficult to reproduce although *E. zuernii* is regarded as the most pathogenic of bovine coccidia (3).

Davis and Bowman (2) reported that of 14 calves they infected with *E. zuernii* three contained no evidence of infection at necropsy and two others were only mildly affected. Marquardt (4) said that only one half in five he fed 500,000 oocysts of *E. zuernii* became clinically ill or produced large numbers of oocysts.

Both the life cycle of *E. zuernii* and the pathogenesis of the disease it causes are poorly understood and the disease must be consistently reproduced to investigate these two aspects. This paper reports on experiments in which the disease was reproduced.

Materials and Methods

Experiment 1

Four Holstein bull calves were obtained within three hours of birth from the Canada Agriculture Research Station, Lethbridge. Colostrum was collected from their dams and fed to the calves for the first three days of their life. Afterwards the calves were fed whole milk for eight weeks and gradually weaned onto a concentrated ration of barley, oats and beet pulp (50:30:15) and grass hay. The calves were kept in individual pens in a small isolated barn. The attendant who fed and cleaned the calves had no contact with other cattle at the institute. Fecal samples from these calves were examined twice weekly for the presence of coccidial oocysts. At the time of infection it was considered that the calves had either no or minimal contact with coccidia as none was ever found in their feces.

At approximately 16 weeks of age calves 3 and 4 were given 1.53×10^5 (low dose) and calves 1 and 2 were given 1.53×10^6 (high dose) sporulated oocysts of coccidia, at least 90% of which were *E. zuernii*, by stomach tube. Beginning 12 days after infection the calves' feces were examined daily for oocysts. All feces passed during the preceding 24 hour period were collected daily from each calf. The feces contained some hay and if of fluid consistency were filtered through a coarse sieve to remove the hay. If the feces were not fluid 2% potassium dichromate was added and the resulting fluid sieved to remove hay. The volume of this fluid was measured. The fluid was then stirred vigorously and five separate aliquots of approximately 20 ml removed and pooled. A sample from this pool was mixed with nine parts of saturated salt solution and five counts of oocysts of this suspension were made using the McMaster technique. The mean number of these counts and the total volume of fecal suspension obtained from a 24 hour collection was used to estimate the total daily oocyst production. This was continued from the 12th to the 61st day after infection.

Experiment 2

Five Holstein bull calves were raised as described for the first experiment. The calves were given 6×10^5 sporulated oocysts of *E. zuernii*, approximately 100% pure, by stomach tube. This assessment of purity was made by examining 500 oocysts from the doses and finding only *E. zuernii*. Two weeks after inoculation with oocysts the calves were transferred to wooden boxes in which they were tied with neck chains to facilitate the daily collection of feces. On the 15th day after inoculation all five calves were given 60 mg of dexamethasone[1] intramuscularly. Feces of the calves

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[1]Azium, Schering Corporation Limited, Pointe Claire, Quebec.

were examined for oocysts as described before with the following differences. From day 12 to day 16 oocysts per gram were estimated using the McMaster technique. Total daily oocyst estimates were made from day 17 to day 30.

Experiment 3

Four Holstein bull calves were raised as described earlier. They were infected and maintained as described in experiment 2. However calves (1 and 3) were given 20 mg of dexamethasone daily on days 12, 15 and 16 after inoculation while calves 2 and 4 were given the same total dose, 60 mg of dexamethasone on day 15 after infection. Feces were examined from day 12 to day 17 by the McMaster technique for oocysts per gram from day 12–day 17. Total daily fecal oocyst estimates were made from day 18–day 30.

Results

The total oocyst counts of *E. zuernii* were recorded in Figure 1 only from day 17 (Exp. 2) and day 18 (Exp. 1 and 3) onwards. Prior to these days only *Eimeria ellipsoidalis* was found in feces. By the 17th day after inoculation few *E. ellipsoidalis* oocysts were seen among the oocysts of *E. zuernii*. The total number of oocysts passed per calf and the mean total per calf in each experiment are shown in Table I. The mean daily total oocyst output of calves from the three experiments is shown in Figure 1; note the tenfold difference in value of the vertical axis between the values for experiment 1 and those of experiments 2 and 3. The peak oocyst output of the calves on days 19 and 20 after infection consisted almost solely of *E. zuernii*. The second peak, seen in the calves of experiment 1 between days 27 and 31 after infection was approximately 70% *E. zuernii* and 30% *E. bovis*.

Experiment 1

All four calves had diarrhea from about the 19th to the 23rd day after infection. The consistency of the feces became very watery and green without the formation of a fecal bolus. A few bright red flecks of blood were seen in the feces of calf 2 on days 20 and 21. Calf 1 had diarrhea for only two days. All calves remained well and ate and drank normally. From the results in Table I there would appear to be no correlation between the size of the dose of oocysts given and the oocyst output. The number of animals involved is so small that no realistic inference could be made in any case.

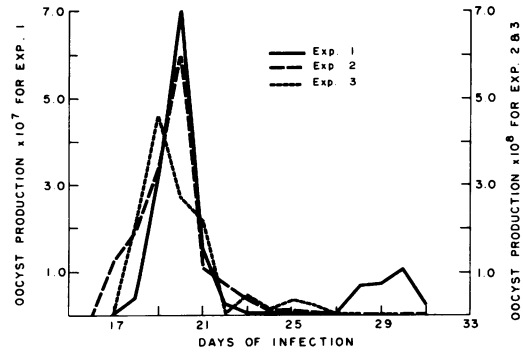

FIGURE 1. Mean daily total oocyst output of *E. zuernii* by calves of experiments 1, 2 and 3.

Experiment 2

All calves developed diarrhea at about the same time as those in the first experiment. However the clinical enteric signs of all the calves changed from diarrhea to dysentery on days 20 and 21 postinfection and, in those calves that survived long enough, reverted back to diarrhea by the 24th day postinfection. After a further 24–48 hours the feces became more solid, appearing almost normal.

The color of the blood passed by these calves was dark red and large amounts of mucus and fibrin or epithelial casts, up to 0.5 m in length, were passed. Calf 4 died on the 20th day and calf 2 on the 25th day after infection.

Experiment 3

The clinical signs seen in these calves were similar to those described in experiment 2. Calf 4 died on the 21st day after infection.

It would appear from Table I that there was a higher yield of oocysts from the calves dosed thrice compared to the calves dosed once with dexamethasone; again the numbers of calves involved are so few that this inference should be given minimal credence.

Discussion

The results reported here confirm Niilo's (6) observations "... the exacerbation of the clinical infection and increased oocyst discharge following treatment with this drug (dexamethasone) ...". The treatment of animals, known to be infected with *E. zuernii*, with dexamethasone can transform a coccidial infection accompanied with mild clinical signs of the disease to a peracute or acute form of coccidiosis in which death may occur. It also appears that treatment with dexamethasone permits an approximately tenfold increase in oocyst output. Presumably the oocyst output of calves treated with the drug more closely

TABLE I

TOTAL AND MEAN TOTAL OOCYST NUMBERS OF *E. zuernii* PER CALF

Experiment	Calf					
	1	2	3	4	5	Mean
1	0.29*H	22.55H	4.44L	33.07L	—	16.09
2	5.55	131.69	1.70	422.13	116.60	135.54
3	161.35T	40.17O	214.21T	48.10O	—	115.95

*All figures $\times 10^7$
H – High dose of *E. zuernii* oocysts (1.53×10^6)
L – Low dose of *E. zuernii* oocysts (1.53×10^5)
O – One injection of dexamethasone (60 mg)
T – Three injections of dexamethasone (3×20 mg)

approximates the full reproductive potential of *E. zuernii* and that in "natural" infections, where there is no treatment, factors such as acquired host resistance act to suppress maximal oocyst production. Niilo (5) suggested that cold may increase the host's susceptibility to clinical coccidiosis and it is possible that both cold and dexamethasone may have a similar effect on the protozoan by directly enhancing the reproduction of the organism in some manner or by suppressing host resistance as mentioned above.

Marquardt (4) pointed out that he reproduced the disease in only one calf in every five infected and Niilo (5) reported that three calves out of eight died from coccidiosis. Both of the above authors used mainly *E. zuernii* as the infecting coccidian and Niilo also injected the animals with dexamethasone. In the same paper Niilo (5) mentioned that the calves died from days 24 to 30 after infection. He used calves that were from five to ten months old and it is possible that the younger age of the calves used in the work described here may have affected the course of the disease; the disease appearing to be more acute as most calves that did die, died earlier.

The difference in success of reproduction of the disease would also appear to be connected with the attempts at maintaining the calves as free from coccidia as possible before infection.

In experiments 1 and 2 peak oocyst production occurred on the 20th day after infection and in experiment 3 on the 19th day. This confirms the observation of Davis and Bowman (1) who stated that "... more calves showed the greatest number of oocysts discharged on the 19th and 20th days than at any other time."

SUMMARY

The production of bovine coccidiosis with *Eimeria zuernii* is described. Treating calves with dexamethasone at a certain stage in the prepatent period of *E. zuernii* appears to alter the progress of the infection so that the disease, rather than merely infection, occurs.

RÉSUMÉ

Les auteurs décrivent la production de la coccidiose bovine avec *Eimeria zuernii*. Le fait de traiter les veaux d'expérience à la dexaméthasone, à un certain stade de la période pré-patente de la coccidie, sembla altérer le progrès de l'infection de façon à provoquer la maladie plutôt que simplement l'infection.

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