Some Pathophysiological Changes Associated with Infection of *Eimeria zuernii* in Calves

P.H.G. Stockdale, A.R. Bainborough, C.B. Bailey and L. Niilo*

ABSTRACT

Twelve Holstein-Friesian calves were divided into two groups, one of which was infected with *Eimeria zuernii*. Fecal oocyst output, weight changes and various blood, cellular, protein and biochemical constituents were examined for both groups. Maximal fecal oocyst output occurred 21 days after infection. Both groups of calves gained weight in a linear fashion until day 21 postinfection when the infected group lost weight rapidly. The packed cell volume of the infected calves appeared markedly reduced. Although there was a reduction in plasma proteins it did not appear significant. There was a significant reduction in plasma Na^+ and Cl^- ions of the infected calves. There were no significant changes in the other blood constituents examined.

RÉSUMÉ

Cette expérience impliquait l'utilisation de deux groupes de six veaux Holstein-Friesian; on infecta le premier avec *Eimeria zuernii* et on utilisa l'autre comme témoin. On détermina le nombre d'oocystes éliminés dans les fèces, les variations de poids, ainsi que certains paramètres sanguins et plasmatiques, tant chez les sujets expérimentaux que chez les témoins. L'élimination fécale d'oocystes atteignit son paroxysme, 21 jours après l'infection. Les sujets des deux groupes accusèrent un gain de poids qui progressa de façon linéaire, jusqu'au 21e jour après l'infection; les sujets du groupe expérimental maigrirent ensuite rapidement. L'hématocrite des veaux expérimentaux accusa une diminution drastique; leurs protéines plasmatiques subirent une baisse qui ne s'avéra cependant pas significative, contrairement à celle des ions Na^+ et Cl^-. Les autres paramètres sanguins vérifiés au cours de l'expérience, ne manifestèrent pas de changements appréciables.

INTRODUCTION

While working with bovine coccidiosis caused by *Eimeria zuernii* we noted that calves appeared to die in three different ways (9). Those that died early in infection (days 18-20 postinfection) had diarrhea and were dehydrated on clinical and postmorten examination. Calves dying later in infection (days 21-25 postinfection) had diarrhea that progressed to dysentery and were both dehydrated and anaemic at ante- and postmortem examination. Calves that survived beyond 25 days after infection either rapidly improved in condition or progressively weakened until they could no longer stand and were then killed.

In the following study, alterations in the levels of some of the blood ions, cells and proteins during the course of the disease were measured.

MATERIALS AND METHODS

Calves were raised and infected as described before (8,10). They varied in age at time of observation from two to four months old. Twelve calves were divided into two equal groups. One group was infected with 9.6×10^6 sporocysts of *E. zuernii* and the second group served as untreated controls. Calves were kept in wooden crates between days 13-30 after infection. All feces passed in 24 hours by each animal were collected daily and the total oocyst output per calf was estimated as described earlier (8).

The animals were weighed and bled, usually twice weekly. Total and differential leucocyte and erythrocyte counts were done with an automatic cell counter (Coulter Counter F.N.). Packed cell volumes (PCV) were measured using a standard microhematocrit technique. Hemoglobin was estimated spectrophotometrically at a wave length of 542 nm. Values for serum Na^+, K^+, Cl^-, CO_2 and blood urea nitrogen (BUN) were obtained using standard methods on a Technicon S.M.A. 4 + 2. Glucose was measured on the same instrument using the glucose oxidase Tinder reaction[1]. Plasma creatinine, uric acid and phosphate were

*Animal Diseases Research Institute, P.O. Box 640, Lethbridge, Alberta (Stockdale and Niilo), Municipal Hospital, Lethbridge, Alberta (Bainborough) and Agriculture Canada Research Station, Lethbridge, Alberta (Bailey). Present address of senior author: Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0.

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[1]Abbott Laboratories, Chicago, Ill.

estimated on a Technicon autoanalyser 2 using standard methods. Plasma total protein values were estimated by the biuret procedure using A-GENT reagent[2]. The reactions were read at 550 nm against a standard prepared from crystalline bovine albumen.

Plasma proteins were separated electrophorectically using Sepraphore[3] cellulose acetate membranes in a Beckman microzone[4] system. Separations were carried out using 0.075M Veronal buffer[5] at pH 8.6 at a constant voltage of 200 volts for 30 minutes. The electrophoretograms were stained with Ponceau S and scanned with a Photovolt integrating densitometer[6]. Albumin concentrations were calculated from total protein after densitometric analysis of the electrophoretograms.

Analyses of variance were carried out to determine whether the variables, Na^+, Cl^- and PCV, responded similarly over the sampling period for the animals within each group (1). The ranges of the response over the sampling period for the variables were determined for each animal and these were compared for the two groups using a "t" test. Polynomial regressions of weight on sampling day were also determined for infected and control calves.

RESULTS

Diarrhea with mucus was observed in two of the six infected calves on day 17 after infection and five of the calves had diarrhea on day 18 after infection. Two of the infected calves had dysentery on day 19 and fibrinous casts were also present in their feces. By day 20 all of the infected calves had diarrhea and dysentery and four of the six had fibrin casts in their feces. Two of these calves died on day 20 after infection but were not obviously clinically dehydrated.

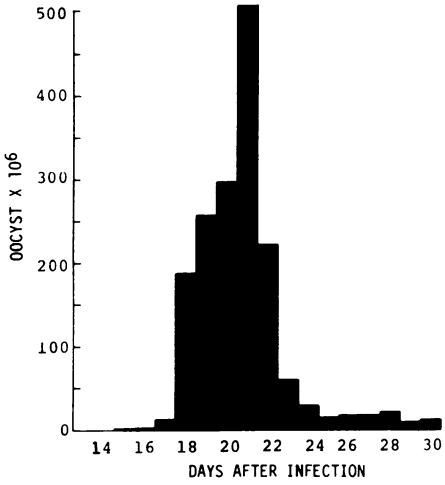

Fig. 1. Mean daily oocyst counts of calves infected with *E. zuernii*.

The degree of dysentery was reduced in two of the remaining four calves on day 22 and in the third calf on day 23 but persisted in the final calf until it died (day 30). Diarrhea continued in all calves after cessation of dysentery until day 25 and then fecal consistency slowly became firmer until day 30. Sick calves continued to eat until shortly before death. The feces of the uninfected calves remained normal throughout.

Thus all data for infected calves after day 20 are calculated on mean values for the four remaining calves. The daily mean oocyst production of calves is given as a histogram in Fig. 1. The day of maximal oocyst output was day 21 after infection. The mean weight gains of the calves are recorded in Fig. 2. The line of best fit was difficult to draw on the data from the control calves. It appears that the control calves gained weight less rapidly after day 24 and this was considered due to their being tethered in the plywood crates. The infected calves gained weight until about day 21 after infection, then lost weight until day 26 and then appeared to regain weight until the end of the experiment.

The line of best fit for the control calves was calculated to be the linear relation: $Y = 3.71 + 0.43x$. The line of best fit for the diseased calves was calculated to be the quadratic relation: $Y = -24.76 + 3.4x - 0.09\chi^2$. Thus the rates of weight gain were significantly different between the two groups of calves.

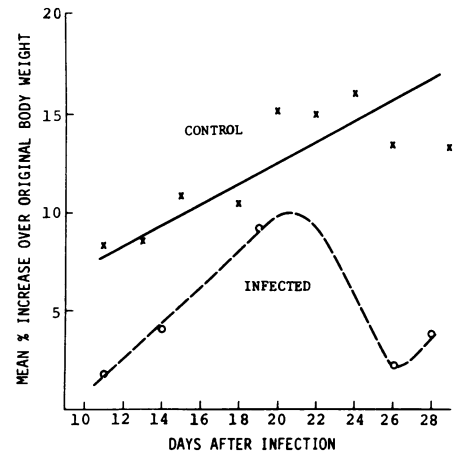

Fig. 2. Mean weight gains of calves infected with *E. zuernii*.

In Figures 3 and 4 the changes in plasma sodium and chloride ions are shown respectively. There was a marked decline in both of these ions in the infected calves beginning about day 18, reaching its lowest point about day 25 and improving slightly by the last day of observation. The difference of the range of readings between the two groups over the experimental

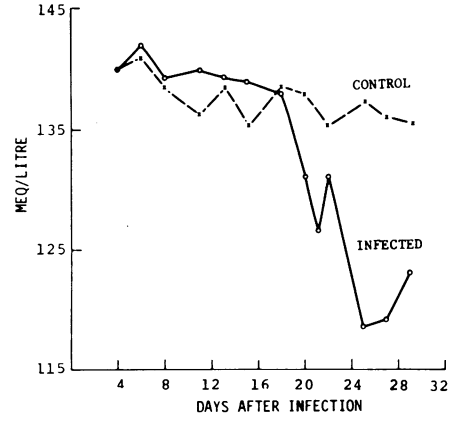

Fig. 3. Mean Na^+ plasma values (MEQ-/litre) of calves infected with *E. zuernii*.

[2]Abbott Laboratories, Pasadena, Calif.
[3]Gelman Instrument Co., Ann Arbor, Mich.
[4]Beckman Instrument Inc., Palo Alto, Calif.
[5]Fisher Scientific Co., Fair Lawn, N.J.
[6]Photovolt Corp., New York, N.Y.

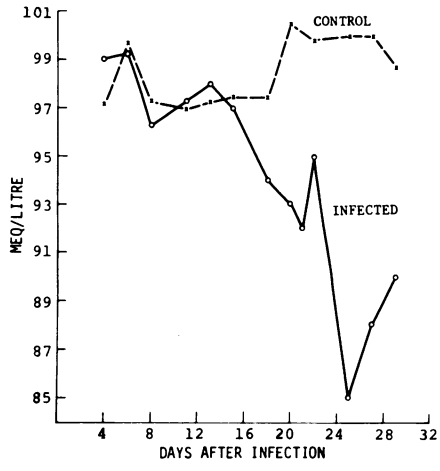

Fig. 4. Mean Cl^- plasma values (MEQ/litre) of calves infected with *E. zuernii*.

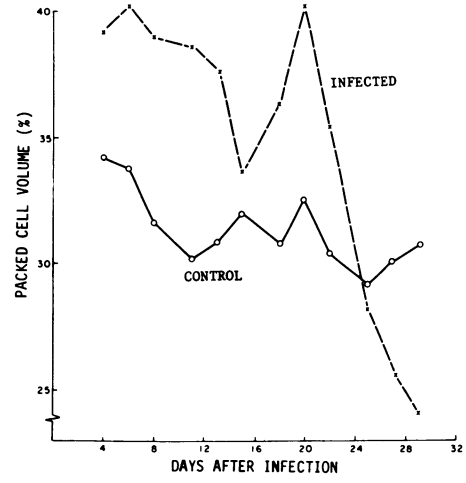

Fig. 6. Mean packed cell volume of calves infected with *E. zuernii*.

period was highly significant for Na^+ ($p < 0.01$) and significant for Cl^- ($p < 0.05$). In Figure 5 the changes in total plasma protein and albumin in infected and control groups are shown. There appeared to be a mild reduction in total plasma protein in the infected calves and this was reflected to a lesser extent in the plasma albumin. The changes in packed cell volumes occurring in both groups of calves are presented in Fig. 6. The packed cell volume of the erythrocytes of the infected calves reached its lowest level on the last day of observation but also appeared to have an earlier nadir on day 15 after infection. The same values in the control calves appeared to remain fairly constant although at a consistently lower level. Similar changes were noted in the hemoglobin levels and numbers of erythrocytes. Thus the lowest mean level of hemoglobin recorded for the infected calves was 9.0 g/dL and the lowest mean level of erythrocytes was 5.5 x $10^6/\mu L$. None of the other parameters examined changed in either infected or control calves during the course of the experiment.

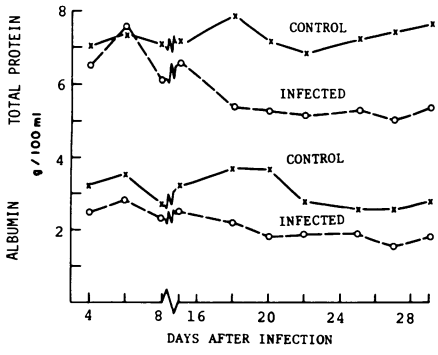

Fig. 5. Mean total plasma protein and mean plasma albumin levels in calves infected with *E. zuernii*.

DISCUSSION

The greatest output of oocysts of *E. zuernii* for this group of calves occurred on day 21 after infection but in six other groups of calves infected with *E. zuernii* in earlier studies maximal oocyst output occurred on day 20 for four groups, day 19 for one and day 21 for another group (8,10). Similar results were reported by Davis and Bowman (2). They found that days 19 and 20 after infection were the days of maximal oocyst production for *E. zuernii*.

Infected and control calves gained weight at approximately the same rate for 20 days after infection. Soon after day 20 the mean weight of the infected group of calves turned abruptly downwards. A similar loss of weight in groups of experimental calves infected with *E. bovis* was reported by Fitzgerald and Mansfield (4,5) and in earlier studies we found a cessation of weight gain in a group of calves infected with *E. zuernii* (11).

Both the sodium ion and chloride ion concentrations of the blood plasma were significantly lowered, with the trend being apparent on day 20 after infection and reaching a nadir on day 25. This is an unusual finding in enteric disease in calves. The lowest values in our calves were 109 mEq/L for Na^+ concentration and 74 mEq/L for Cl^- concentration. These values are much lower than those seen in severe neonatal calf diarrhea where the lowest and mean values for Na^+ and Cl^- were 120 (138) and 90 (102) mEq/L respectively (12). The difference is probably explicable because of the difference in site of the lesions in the two diseases. Neonatal calf diarrhea is usually a disease of the small intestine while the lesions of coccidiosis due to *E. zuernii* are found in the cecum and proximal colon. The colon is the major site of conservation of intestinal sodium and chloride ions and relatively minor impairment of large bowel function can lead to significant increases in both fecal water and electrolyte loss (7). Fitzgerald (3) noted only minor changes in serum Na^+ in calves infected with *E. bovis* unless the clinical signs were severe and then he recorded levels as low as 90.0 mEq/L. He also noted an increase in serum potassium in severely affected calves; a change which did not occur in our calves. Recently it has been reported that depression of Na^+ and Cl^- levels occur in calves infected with *E. zuernii* and that the lowering of these ions may contribute to the nervous signs sometimes associated with bovine coccidiosis (6).

The mean total plasma protein and mean plasma albumin levels appeared mildly but not significantly affected in the calves of this experiment. This reduction appears to be less than that reported in infections of calves with *E. bovis* (4).

There were marked decreases in the packed cell volumes, hemoglobin levels and numbers of erythrocytes in the infected calves. As can be seen from Fig. 6, the mean PCV values for the two groups of calves were markedly different at the start of the experiment. By day 24 the mean PCV value of the

infected calves had dropped to the same as that of the control group and continued to drop to below the value of the control group. These differences between the groups were not statistically significantly different but it can be seen from the graph that while there was a slow and slight decline in PCV values for the control calves there was an abrupt and marked decline from day 20 to day 29 in the infected group. This sharp and pronounced decline was also coincidental with the damage to, and removal of, the epithelium of the large intestine (9). Similar changes in these parameters have been noted by others (6). This is in marked contrast to the disease caused by *E. bovis* in which an increase in hemoglobin and packed cell volume is reported (4).

The marked reduction in Na^+ concentration is probably associated with a reduction in plasma water and possibly a hypovolemia. This in turn should be accompanied by an apparent increase in packed cell volume and plasma protein. Thus it is possible that the reduction in packed cell volume and the slight lowering of plasma protein levels that are recorded here are poor indicators of the true degree of the change in these two parameters.

We conclude that just prior to the peak oocyst output there is maximal loss of large bowel epithelium due to the destruction of epithelial cells by second generation schizogony and gametogony of *E. zuernii* (9). With this destruction of the epithelium there is a reduction of reabsorption of water, Na^+ and Cl^- from the intestinal contents. The abrupt loss of weight and reduction of the plasma concentration of these two ions support this contention. As the epithelium is lost, capillaries of the large intestinal lamina propria are exposed and rupture leading to loss of erythrocytes and plasma which again is partially confirmed by our results here (packed cell volume and total plasma protein) and earlier (9).

From these and earlier results (9, 11) we postulate that calves dying earlier in infection (days 18-21) probably die from dehydration while those dying later (days 21-25) probably die from a combination of dehydration and blood loss. Most calves then recover from the disease and as can be seen from Figs. 2, 3, 4 and 5 there does appear a trend to normal values. The lack of such a trend in Fig. 6 is probably due to the fact that even with increased erythrocyte production the packed cell volume takes longer to return to normal.

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REFERENCES

1. **BLISS, C.I.** Statistics in Biology. Volume II. p. 639. New York: McGraw-Hill. 1970.
2. **DAVIS, L.R. and G.W. BOWMAN.** Coccidiosis in cattle. Proc. U.S. Livestock Sanit. Ass. pp. 39-50. 1951.
3. **FITZGERALD, P.R.** Effect of bovine coccidiosis on blood serum sodium and potassium levels of calves. Am. J. vet. Res. 28: 667-670. 1967.
4. **FITZGERALD, P.R. and M.E. MANSFIELD.** Effects of bovine coccidiosis on certain blood components, feed consumption and body weight changes of calves. Am. J. vet. Res. 33: 1391-1397. 1972.
5. **FITZGERALD, P.R. and M.E. MANSFIELD.** Efficacy of monensin against bovine coccidiosis in young Holstein-Friesian calves. J. Protozool. 20: 121-126. 1973.
6. **KEMP, R.L. and D.D. SUSIE.** Clinical pathology of *Eimeria zuernii* coccidiosis in calves. Abs. 53rd a. Meet. Am. Soc. Parasit. p. 78. 1978.
7. **PHILLIPS, S.F. and J. GILLER.** Thecontribution of the colon to electrolyte and water conservation in man. J. Lab.clin. Med. 81: 733-746. 1973.
8. **STOCKDALE, P.H.G.** Production of coccidiosis using oocysts, sporocysts and sporozoites of *Eimeria zuernii, E. contorta* and E. *nieschulzi.* Can. J. Zool. 54: 1064-1068. 1976.
9. **STOCKDALE, P.H.G.** The pathogenesis of the lesions produced by *Eimeria zuernii* in calves. Can. J. comp. Med. 41: 338-344. 1977.
10. **STOCKDALE, P.H.G. and L. NIILO.** Production of bovine coccidiosis with *Eimeria zuernii.* Can. vet. J. 17: 35-37. 1976.
11. **STOCKDALE, P.H.G. and W.D.G. YATES.** Resistance to *Eimeria zuernii* produced after chemotherapy of experimental infections in calves. Vet. Parasit. 4: 209-214. 1978.
12. **TENNANT, B., D. HARROLD and M. REINA-GUERRA.** Physiologic and metabolic factors in the pathogenesis of neonatal enteric infections in calves. J. Am. vet. med. Ass. 161: 993-1007. 1972.