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Distribution of cryptosporidia within the gastrointestinal tract of young calves

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Natural cryptosporidial infection of the gastrointestinal tract was recognised in four young calves by light microscopy and correlated with scanning and transmission electron microscopy in two of the calves. Seven to 10 sites of the small intestine were examined in each calf and cryptosporidia were most numerous in the posterior 50 per cent. None were found in the anterior 20 per cent of the small intestine, the abomasum or the colon, but they were present in the mucosa of the caecum of three calves.

CRYPTOSPORIDIA are protozoan parasites which are associated with the intestinal mucosa of a wide variety of animal species including man (Pohlenz et al 1978a). In cattle they were first recognised in an eight-month-old heifer in the USA (Panciera et al 1971). Since then they have been found on a number of occasions in calves up to three weeks old in the USA (Meuten et al 1974, Schmitz and Smith 1975, Powell et al 1976, Pohlenz et al 1978b), Canada (Morin et al 1976), Australia (Barker and Carbonell 1974) and the United Kingdom (Pearson and Logan 1978, Logan et al 1979, Snodgrass et al 1980). The purpose of this paper is to examine the distribution of cryptosporidia in the small intestine of naturally infected calves as demonstrated by light, scanning and transmission electron microscopy.

Materials and methods

Animals

Results were obtained from four calves. Two colostrum-deprived calves (1 and 2) were taken from a local farm within 12 hours of birth and kept in isolation in individual raised crates at the laboratory. On arrival at the laboratory they were given 4 g of an IgM-rich fraction of bovine serum intravenously (Logan and Penhale 1972) and subsequently fed three pints of milk substitute twice daily. They were intended for use in experimental enteric infections but developed spontaneous diarrhoea when five and seven days old respectively without receiving any experimental challenge. The remaining two calves (3 and 4) were taken from separate farms where diarrhoea was a problem. The calves were 14 and 10 days old respectively and had been diarrhoeic for several days when examined.

All four calves were anaesthetised using pentobarbitone and seven to 10 samples were collected throughout the length of small intestine from the duodenum to the ileum, as previously described (Pearson et al 1978). Samples of the small intestine from all calves were processed for histopathology and additional samples were collected from calves 1 and 4 for scanning and transmission electron microscopy. After samples of abomasum, caecum and colon were collected for histopathological examination the calves were killed with an overdose of pentobarbitone.

Light microscopy

Samples of the gastrointestinal tract were pinned onto cork and placed immediately in 10 per cent buffered formalin. Sections were cut at 6 μm and stained by the haematoxylin and eosin and Giemsa methods.

Scanning electron microscopy

Samples were taken from the anterior, middle and posterior small intestine of calves 1 and 4 at the same time as those for light microscopy. Small blocks of tissue, 0·5 cm diameter, were placed in a fresh solution of two parts glutaraldehyde to one part osmium tetroxide at 4°C for two hours. They were dehydrated in a graded series of ethanol from 70 to 100 per cent and stored at 4°C in amyl acetate until required. The tissues were dried in a critical point drying apparatus. They were attached to aluminium stubs with adhesive, coated with gold and examined in an ISI 60 scanning electron microscope.

Transmission electron microscopy

Portions of the anterior, middle and posterior

small intestine were also collected at the same time from calves 1 and 4 and placed immediately in 4 per cent glutaraldehyde in sodium cacodylate buffer. They were post fixed in 1 per cent osmium tetroxide and embedded in Araldite. Ultrathin sections were stained with lead citrate and uranyl acetate and examined in a Philips 301 electron microscope.

Microbiological studies

Swabs from the anterior, middle and posterior small intestines and the caecal contents were incubated overnight on blood agar and enrichment techniques were employed for salmonellae. Isolates of *Escherichia coli* were tested against a range of antisera (Logan et al 1979).

Caecal contents were examined for viruses by direct electron microscopy using method C of McNulty et al (1979).

Results

Microscopic findings

By light microscopy, cryptosporidia were found in the intestinal tracts of all calves (Fig 1). The organisms were more clearly seen in sections stained by Giemsa than by haematoxylin and eosin. The distribution of the organisms within the intestinal tract is shown in Fig 2. In all four calves they were found on the villi at several sites in the small intestine. The majority of the organisms were located in the posterior half of the small intestine but smaller numbers were present anterior to this. However, organisms were not present in the anterior 20 per cent of the small intestine. Villi in the affected regions were blunted, thickened and occasionally fused (Fig 3). By scanning electron microscopy, large numbers of organisms were seen coating the villi in the posterior small intestine (Fig 4) where they were either embedded within the microvillous border or lying free on the surface. Craters were frequently observed in the villous surface from which organisms had presumably been removed either by natural shedding or fixation.

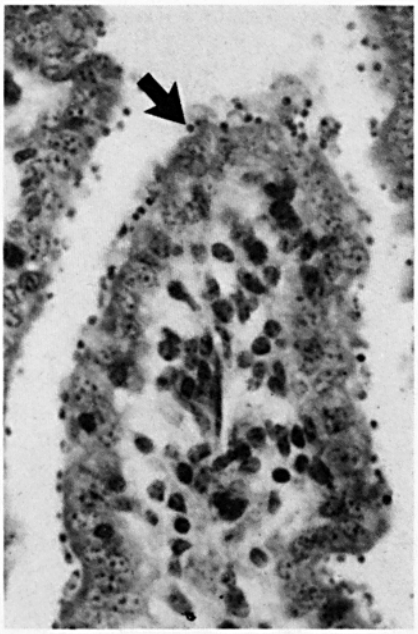

FIG 1: Villus tip, posterior small intestine. Cryptosporidia (arrow) coat the microvillous surface of epithelial cells. H&E ×600

By transmission electron microscopy trophozoites and schizonts were observed frequently in intimate association with the surface of the epithelial cells (Fig 5). Occasionally gametes were seen but oocysts were detected only infrequently. Schizonts were also found lying free within the lumen. The outer envelope of some schizonts had a crenated appearance associated with small everted pouch-like

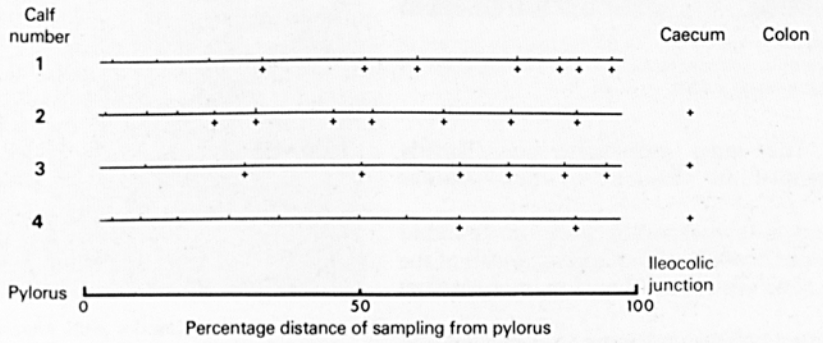

FIG 2: Distribution of cryptosporidia in the intestinal tract of calves

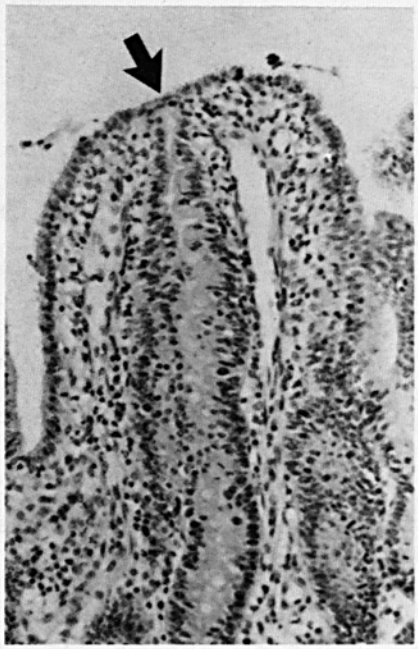

FIG 3: Blunted villi showing fusion (arrow) associated with cryptosporidia in the distal small intestine. H&E ×150

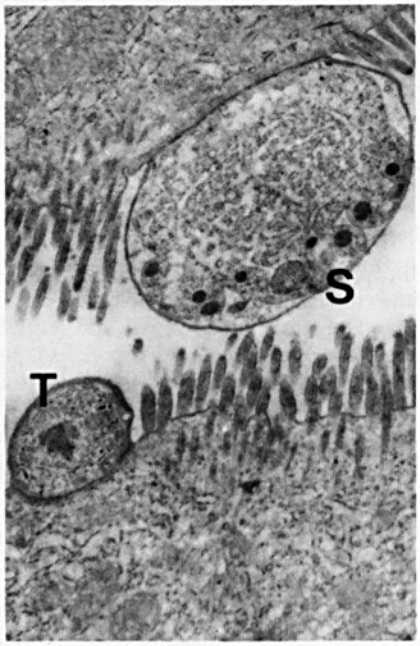

FIG 5: A trophozoite (T) and schizont (S) in the microvillous brush border of epithelial cells. Magnification ×11,000

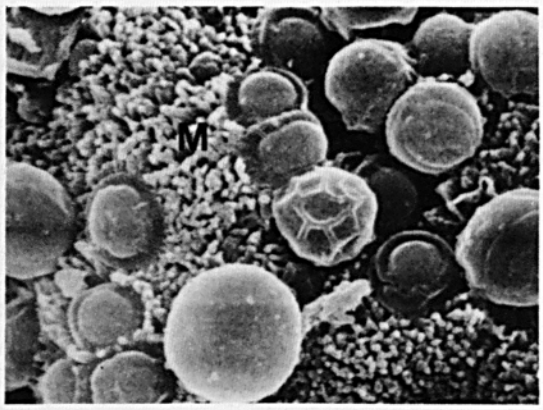

FIG 4: Cryptosporidia associated with the microvillous surface of villous epithelial cells, posterior small intestine. Microvilli (M) are prominent. Magnification ×5000

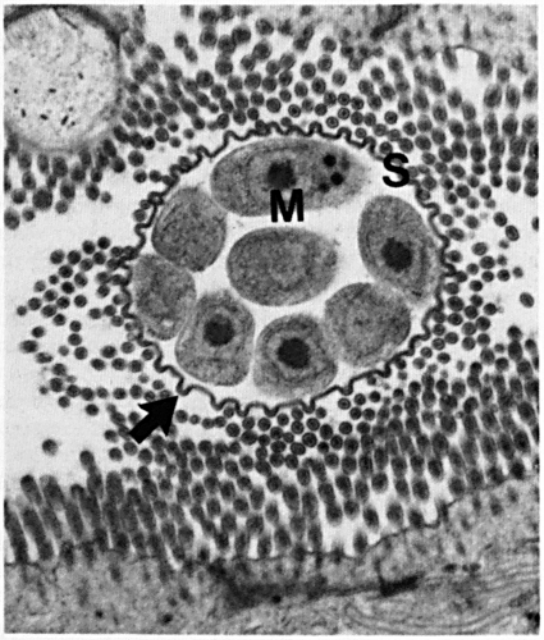

FIG 6: Schizont (S) contains eight merozoites (M). The outer envelope has a crenated appearance associated with everted pouches (arrow). Magnification ×12,000

structures. The outer membrane was slightly thickened around the curvature of these pouches (Fig 6).

In three calves cryptosporidia were also found in the microvillous brush border of epithelial cells of the caecum but none was seen in the colon or abomasum of any calf.

Bacteria were not seen adhering to the mucosa at any site by any of the techniques employed and

viruses were not observed in epithelial cells in calves 1 and 4 at any site examined.

Microbiological findings

E coli were recovered from all samples examined but were not typable by the range of antisera used.

Rotavirus was detected in the caecal contents of calf 1 and coronavirus in calf 2. Viruses were not detected in the remaining two calves.

Discussion

Cryptosporidia were found predominantly in the posterior half of the small intestine of calves in association with pathological lesions. While there are several reports of natural cryptosporidiosis in the small intestine of neonatal calves (Barker and Carbonell 1974, Meuten et al 1974, Schmitz and Smith 1975, Powell et al 1976) sufficient samples were not collected to enable a precise distribution pattern to be obtained. The results of the present study are in close agreement with those of Morin et al (1976) and Pohlenz et al (1978b) who examined five sites of the small intestine.

The presence of cryptosporidia within the large intestine of calves is variable. While some workers (Meuten et al 1974, Pohlenz et al 1978b) found them in the colon, others were unable to demonstrate them at this site (Schmitz and Smith 1975, Morin et al 1976, Pearson and Logan 1978). In the present study cryptosporidia were not found in the colon but were seen in the caecum of three animals. However, only one site of each colon was collected and their presence may have been missed. The caecum has not previously been reported as a site in natural cryptosporidiosis in the calf although it has been recognised in experimentally challenged calves (Moon and Bemrick 1981) and foals (Snyder et al 1978).

The corrugated appearance of some of the schizonts was similar to that reported in cryptosporidiosis in rabbits (Inman and Takeuchi 1979).

The location of cryptosporidium predominantly within the posterior small intestine may be of significance in relation to its enteropathogenicity. It is known that there is a net absorption of fluid from the posterior small intestine (Bywater and Logan 1974) and it seems likely that damage to this area contributes to the increased loss of water which occurs in calves with diarrhoea. This is supported by studies with enteropathogenic *E coli* (Pearson et al 1978, Bellamy and Acres 1979) in which pathological lesions in the calf's posterior small intestine were associated with profuse diarrhoea. In contrast, lesions of similar severity in the anterior small intestine associated with rotavirus infection (Logan et al 1979) resulted in only mild diarrhoea. Thus the distribution of a particular pathogenic organism may be critical in the aetiology of diarrhoea in young calves.

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