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CRYPTOSPORIDIOSIS IN TANZANIAN GOAT KIDS: SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC OBSERVATIONS

By

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MATOVELO, J. A., T. LANDSVERK and G. AMAYA POSADA: *Cryptosporidiosis in Tanzanian goat kids. Scanning and transmission electron microscopic observations.* Acta vet. scand. 1984, *25*, 322—326. — Trophozoites, gametes, and schizonts characteristic of cryptosporidia were demonstrated in 2 Tanzanian goat kids. In a heavily infected kid the parasite was associated with villous atrophy and crypt hyperplasia. An enhanced epithelial cell ageing and extrusion rate, possibly due to a drain on cell metabolites by the parasite, is suggested. The protozoan seemed to induce host cell specializations, including parasitophorous envelope and development of smooth-membraned vesicles. The vesicles possibly have a function in the transport of nutrients from the host cell to the parasite.

cryptosporidia; parasitophorous envelope; villous atrophy; epithelial extrusion; cytoplasmic vesicles.

The cryptosporidia, which are coccidia of the suborder Eimeriorina and which seem to constitute a single species genus (*Tzipori et al.* 1980), are progressively appearing to be more important as a cause of diarrheic condition in animals (review, see *Angus* 1983). They parasitize the intestinal epithelial cells and may be associated with morphological changes in the intestinal mucosa (*Panciera et al.* 1971, *Meuten et al.* 1974, *Moon et al.* 1978, *Tzipori et al.* 1983). Although cryptosporidiosis has been reported in goats by others (*Mason et al.* 1981, *Tzipori et al.* 1982), the present paper gives the first account of the condition

in Tanzanian goat kids. Marked intestinal lesions ware associated with the infection. Features of host-parasite relationship are emphasized.

MATERIALS AND METHODS

The cases of cryptosporidiosis were found in an experimental material comprising 7 six-week-old local breed goat kids, reared at the University farm, Morogoro, Tanzania. The experiment, which included surgical isolation of Peyer's patches in kids, will be published separately, but for this report some selected data will be given. The cryptosporidia were found in ileal enterocytes of 2 kids. The kids concerned, one male and the other female, suckled their mothers and had free access to hay and water. The ileal biopsies were obtained by laparatomy under anesthesia with Rompun® 0.2 mg/kg and Ketalar® 11 mg/kg. The biopsies were fixed in 0.9 % glutaraldehyde and 0.7 % paraformaldehyde in a 0.14 mol/l cacodylate buffer.

Specimens for light microscopy (LM) were dehydrated in ethanol, embedded in paraffin, sectioned about 5 μ and stained with hematoxylin and eosin (HE).

Specimens for scanning electron microscopy (SEM) were dehydrated in acetone, critical point dried with carbon dioxide as the transitional fluid, attached to metal stubs with silver paste and coated with gold in a vacuum evaporator. Coated samples were examined in a Jeol 50A scanning electron microscope with an accelerating voltage of 10—15 KV. Photographs were recorded on a Polaroid type 52 film.

Specimens for transmission electron microscopy were post-fixed in 2 % cacodylate buffered osmium tetroxide for 2 h, dehydrated in acetone and embedded in Epon. The thin sections were made with a diamond knife and stained with uranyl acetate and lead citrate.

RESULTS

The kids concerned showed no sign of diarrhea before or at the day of biopsy. Examination of feces revealed moderate numbers of Eimeria oocysts.

LM showed a heavy infection in one of the kids, with numerous round bodies studded in the brush border. The epithelium was low and irregular, villi were atrophic and crypts

hyperplastic (Figs. 1—3). The other kid had a slight infection; its mucosa was not markedly changed.

SEM of the heavily infected kid confirmed the villous atrophy and showed numerous parasites in the brush border (Figs. 5—7). The organisms varied in size, apperently corresponding to developmental stages. Younger stages were rather smooth, except for a series of vertical grooves along their attachment to the apical plasma membrane. Mature schizonts were recognized by their characteristic surface contours impressed upon the parasitophorous envelope by the merozoites (Fig. 7). Empty shells or craters seemed to be remnants of schizonts that had released their contents (Fig. 6).

TEM showed the various stages of parasite development, including trophozoites, gametocytes and schizonts (Figs. 8—11). Macrogametes were recognized by their polysaccharide granules (Fig. 11), whereas schizonts showed characteristic elongated merozoites. Early trophozoite development seemed to include incorporation of the parasite into a pocket of the apical cell membrane (Fig. 8), formed by remodelling of the microvillous zone. Later in the development, a folded membranous structure (feeder organelle) connected the host cell plasma membrane with the parasite. Development of feeder organelle and endoplasmic reticulum of the trophozoites coincided with the occurrence of numerous smooth-membraned vesicles in apical host cell cytoplasm (Fig. 10). Cytolysis and extrusion of epithelial cells seemed enhanced, some of the necrotic cells being parasitized. Merozoites resembling those of Eimeria spp. were occasionally seen intracellularly in the epithelium. Phagocytosis of the parasites by migrating neutrophils was observed (Fig. 12).

DISCUSSION

The morphology and developmental stages of the protozoan described in the present paper correspond to those of cryptosporidia (*Vetterling et al.* 1971, *Pohlenz et al.* 1978, *Pearson & Logan* 1983). Cryptosporidiosis appears to be a condition of world-wide importance, and the present report seems to be the first one on the condition in a ruminant from Tanzania.

The host-parasite relationship in cryptosporidiosis seems to involve induction of specific responses in the host cell by the parasite. The responses include enfolding of the parasite into a host-

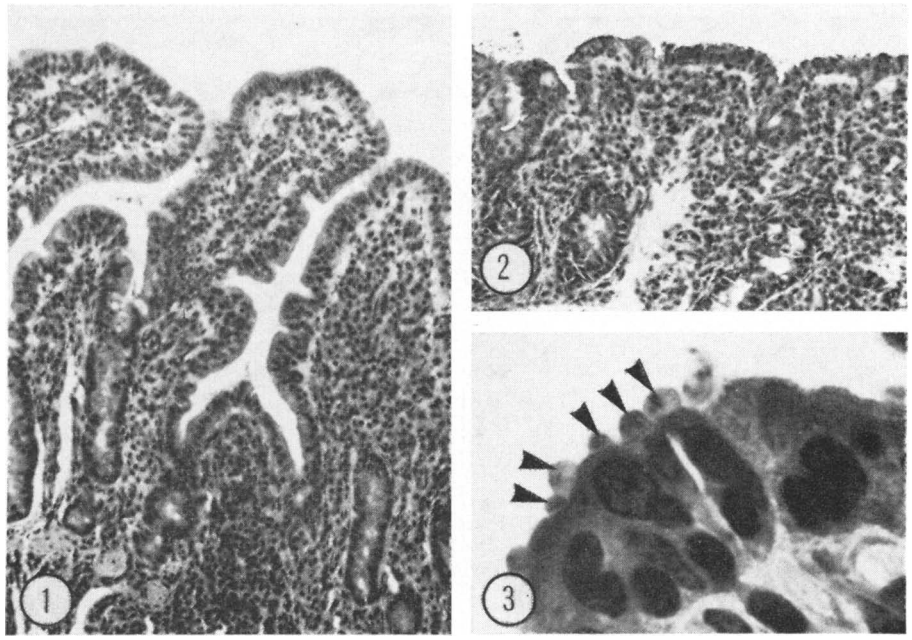


Figure 1. Ileal mucosa in non-infected goat kid showing villi of normal appearance. HE, × 160.

Figure 2. Ileal mucosa from heavily infected kid. Villous atrophy is almost complete and epithelial lining is uneven, corresponding to irregular organization of epithelial cells and the occurrence of cryptosporidia. HE, × 160.

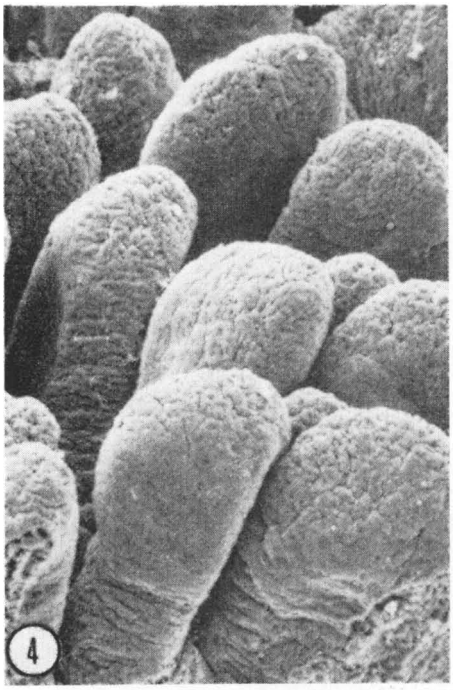
Figure 3. Ileal mucosa fram a heavily infected kid. Various sizes of of cryptosporidia (arrows) within the epithelial brush order. HE, × 1,600.

Figure 4. Scanning electron micrograph, ileum of control goat kid. Tongue-shaped villi with narrow furrows. × 650.

Figure 5. Scanning electron micrograph, ileum of severely affected kid. Villi are stunted and fused (arrows). Numerous small spherical cryptosporidia are seen at the surface. × 300.

Figure 6. Scanning electron micrograph. Numerous cryptosporidia of various sizes within the brush border. Protruding epithelial cells devoid of microvilli (E) seem to be extruded. Arrow indicates a probable neutrophil. × 1,500.

Figure 7. Scanning electron micrograph. Cryptosporidia of various sizes corresponding to different maturation stages: the smaller bodies correspond to younger stages. Vertical grooves are seen at the insertion of the parasitophorous envelope to the luminal cell membrane (G). A mature schizont (S) is recognized by merozoites visible through the parasitophorous envelope. × 4,500.



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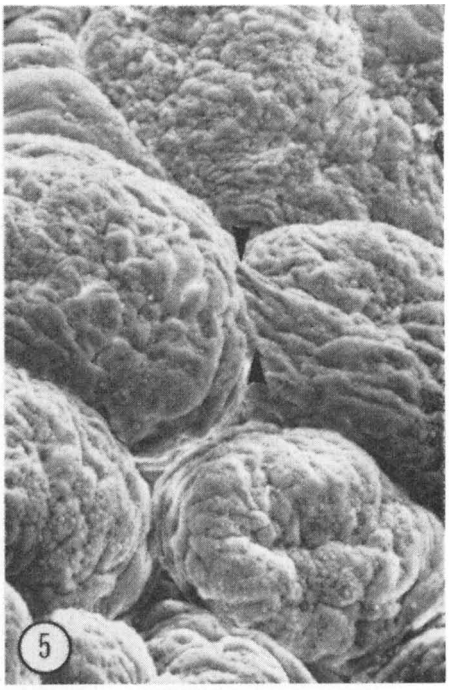

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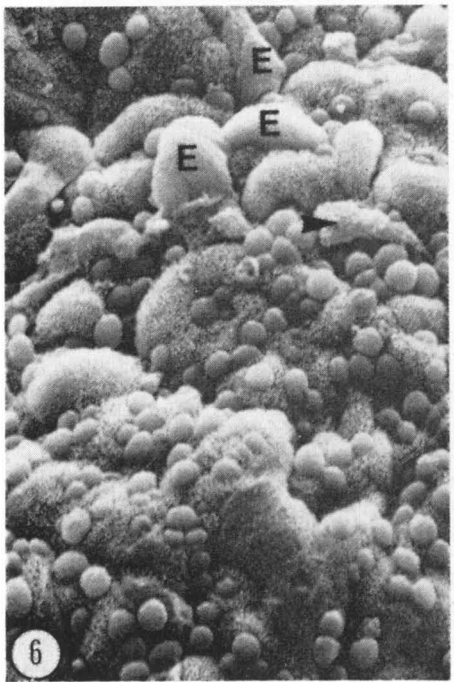

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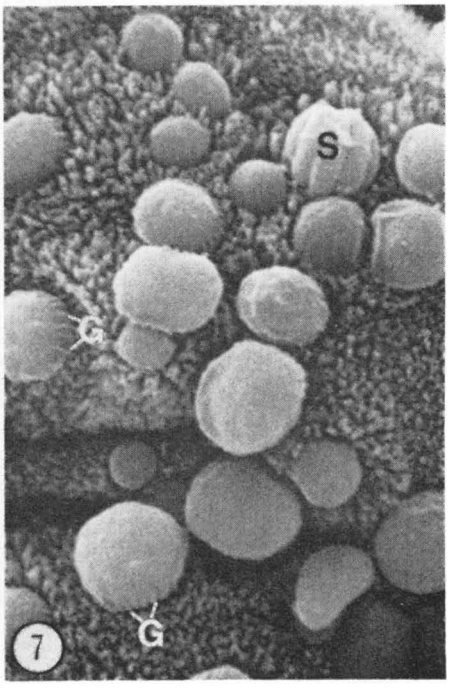

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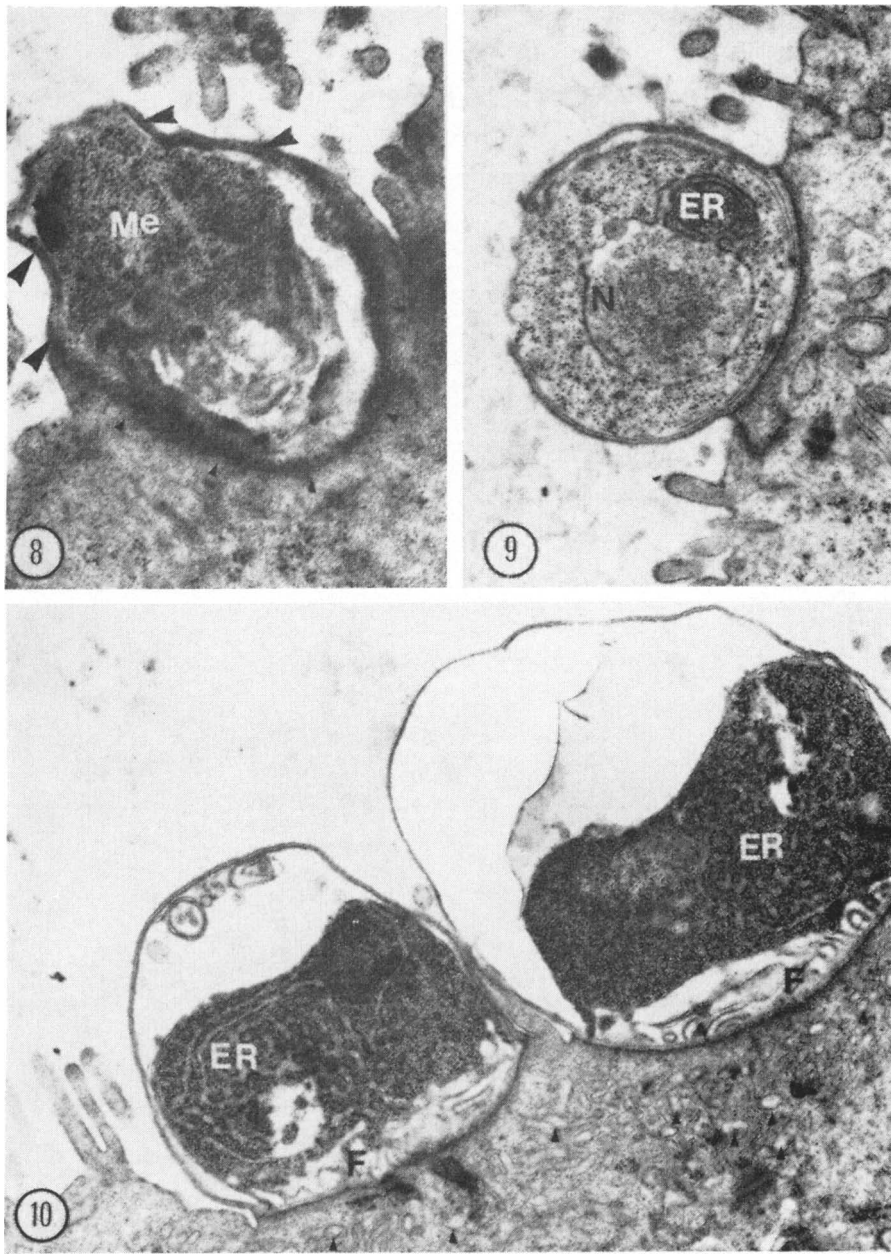
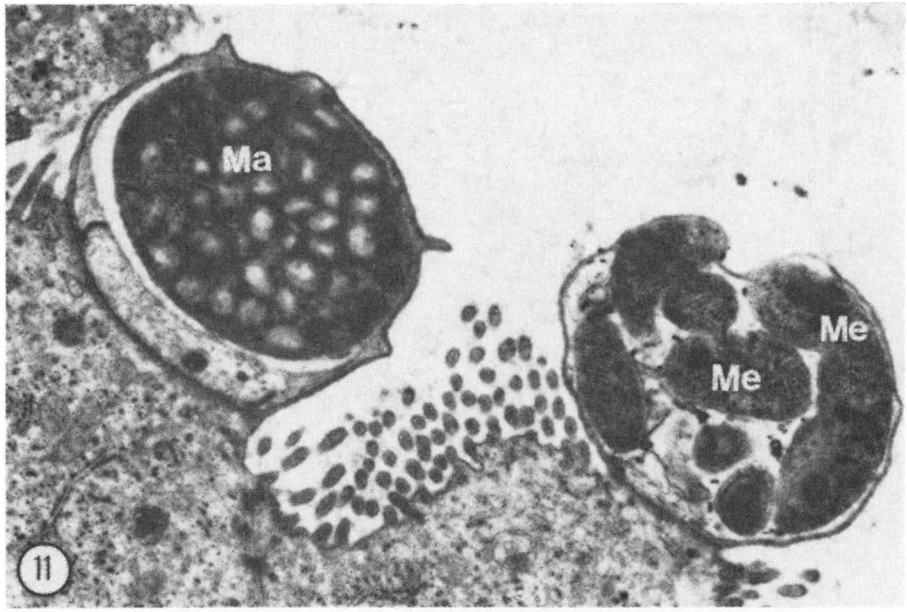


Figure 8. Merozoite (Me) developing into a trophozoite after attachment to the host cell. Small arrows indicate developing attachment zone. Microvilli or extensions of the luminal host cell (large arrows) are seen in the process of enveloping the parasite. × 30,000.

Figure 9. Trophozoite with nucleus (N), endoplasmic reticulum (ER), and incomplete parasitophorous envelope. × 23,000.

Figure 10. Trophozoites with extensive endoplasmic reticulum (ER), feeder organelle (F) in the attachment zone, and smooth-membraned vesicles (arrows) in the adjcent host cell cytoplasm. × 20,000.



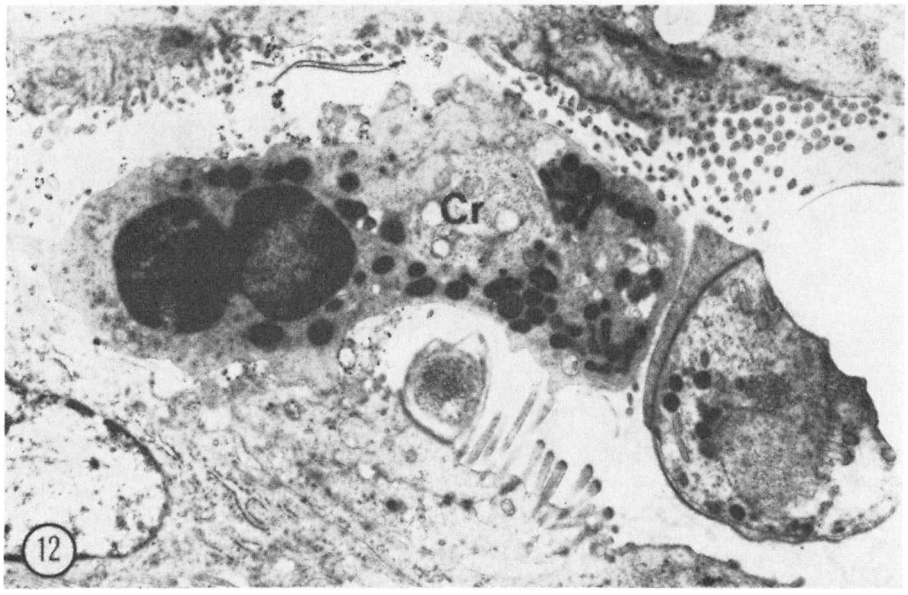


Figure 11. Macrogamete (Ma) with polysaccharide granules and a mature schizont with merozoites (Me). × 13,500.

Figure 12. A neutrophil with an engulfed cryptosporidium (Cr) and in the proximity of 2 other cryptosporidia.

cell-derived envelope. Our findings in this context are in agreement with the concept of intracellular location of the parasite, as originally pointed out by *Hampton & Rosario* (1966) and later confirmed by *Vetterling et al.* (1971) and *Pearson & Logan* (1983). The occurrence of smooth-membraned vesicles in the host cell cytoplasm adjacent to the attachment zone may be another specific response. The vesicles, which possibly correspond to the vesicles suggested as endocytic by *Vetterling et al.* 1971), were found abundantly at the stage of maximal protein synthesis in the parasite, as judged from the development of endoplasmic reticulum. The vesicles may be a host-cell counterpart to the feeder organelle, serving in transport of nutrients to the parasite. The parasite, although probably not so harmful to the host cell in the early stages of development, nevertheless seemed to be associated with villous atrophy and crypt hyperplasia, a finding in agreement with other reports (*Panciera et al.* 1971, *Meuten et al.* 1974, *Moon et al.* 1978, *Tzipori et al.* 1983). Such villous atrophy is normally associated with enhanced epithelial cell extrusion, indicated in this case by the scanning electron micrographs. Overt cytolytic changes were not associated with the parasite. However, the parasite may have caused a drain on the host cell metabolites thus enhancing the normal process of senescent epithelial cell extrusion. The consequences of parasitism are apparently related to severity of infection, as judged from the absence of marked villous atrophy in the kid with slight infection.

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SAMMENDRAG

Kryptosporidiose hos Tanzanianske kje. Scanning- og transmisjonselektronmikroskopiske funn.

Hos Tranzanianske kje såes trophozoiter, gameter og schizonter med morfologi typisk for kryptosporidier. Hos ett dyr med massiv infeksjon fantes atrofi av tarmtottene og hyperplasi av kryptene. Et forkortet livsløp og forøket avstøtning av epitelcellene i tarmen ble satt i forbindelse med parasittenes forbruk av cellemetabolitter. Protozoene syntes å forårsake spesialiseringer hos vertscellen, bl. a. vakuoler som omgav parasittene og cytoplasmatiske vesikler. Vesiklene har kanskje betydning i transporten av metabolitter fra vertscellen til parasitten.

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