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A Light and Electron Microscopic Study on Abomasal Globidiosis in Somali Goats

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Abdurahman, O. S., M. Hilali and B. Järplid: A light and electron microscopic study on abomasal globidiosis in Somali goats. Acta vet. scand. 1987, 28, 181–187. – Abomasum from apparently healthy Somali goats with globidiosis showed pinhead sized nodules embedded in the mucosa. The nodules consisted of encapsulated cysts, containing mature or immature schizonts. Glandular atrophy and lymphohistiocytic cell reaction were often found in the vicinity of these cysts. The fine structure of immature and mature cysts is described in details. The mature cysts contained elongated, spindle shaped merozoites (type I) or shorter, ovoidal merozoites (type II). Some mature cysts also had basophilic granular bodies among the merozoites. Type I and type II merozoites were morphologically different from those earlier described in goats.

abomasum; globidium; ultrastructure; histopathology.

Introduction

Globidium cyst-like bodies from the abomasum and small intestine of sheep and goats have been reported under the name of Globidium gilruthi (*Chatton* 1910). These cysts were later considered to be schizonts of Eimeria (*Levine* 1973). In sheep, the fine structure of these cysts have been described (*Hilali* 1973, *Mehlhorn & Heydorn* 1976, *Porchet-Hennere* 1977, *Hilali & Scholtyseck* 1979, *Sénaud et al.* 1984).

Globidium infection in goats has been reported from different African countries such as South Africa (*Canham* 1931), Egypt (*Soliman* 1958), Sudan (*Soliman* 1960), Tanzania (*Bwangamoi* 1968) and Kenya (*Mugera & Bitakaramire* 1968). The fine structure of goat globidium has been described by *Mehlhorn et al.* (1984) from West Germany.

The aim of this work was to give a light and electron microscopic description of globidium cysts from the abomasum of Somali goats and to compare it with previous descriptions of sheep and goat globidium. The tissue reaction around the cysts containing giant schizonts was also studied by light microscopy.

Materials and methods

The abomasum of 55 apparently healthy and normally slaughtered goats at the main slaughter-house in Mogadishu was opened longitudinally and thoroughly inspected for the presence of globidium nodules. Suspected nodules were dissected for light and electron microscopy. Specimens for light microscopy were fixed in 10 % formaldehyde solution, embedded in paraffin, secti-

oned at about 5 µm and stained with hematoxylin and eosin (HE). In some cases van Gieson's stain, PAS and methylgreen and pyronin (*Kurnick* 1955) were also used.

Specimens for electron microscopy were fixed in a mixture of 3 % glutaraldehyde and 3 % paraformaldehyde in 0.2 mol/l phosphate buffer (pH 7.4). They were kept in +4°C until air-freighted to Uppsala, Sweden, where they were washed in 0.1 mol/l phosphate buffer (pH 7.4), postfixed in 1 % OsO_4 for 1 h at +4°C, dehydrated in graded series of ethanol and embedded in Epon. Semithin sections were stained with toluidine blue for light microscopy. Ultrathin sections were cut with diamond knives, using LKB Ultramicrotome, mounted on copper grids, stained with uranyl acetate for about 20 min and then with lead citrate for 2 min. The specimens were examined under a Philips EM 420.

Results

Macroscopic examination

The abomasum of 19 animals showed pinhead sized whitish nodules embedded in the mucosa and sometimes bulging from its surface. Some of these nodules were ruptured and looked like small crater-shaped erosions with raised edges. There were no visible changes in the mucosa surrounding the nodules. The number of globidian nodules in each abomasum varied from 2 to numerous. They were usually scattered all over the mucosa. However, they were more frequently seen in the fundic than in the pyloric region. They also occurred more in the folds than between the folds.

Light microscopic examination

The globidium cysts were embedded at varying depth in the mucosa. Most of them were located in the central part of the lamina propria but in some cases the cysts were lying more adjacent to the muscularis mucosa. Sometimes the cyst occupied the main depth of the lamina propria causing bulging of the mucosal surface (Figs. 1 and 2).

The cysts were roughly spherical or ovoidal in shape, varying in size from 230 × 270 µm to 420 × 550 µm. They had a two-layered wall. The inner layer was in some cases homogenous and in other cases somewhat fibrillar and vacuolated. It stained more eosinophilic in HE than the outer one. This outer layer often consisted of a rim of fimbriae (Fig. 3).

The cysts were in different stages of development. They were either immature or mature. The immature forms contained either multinucleated schizonts or schizonts with developing merozoites. There were 2 types of mature cysts. One contained elongated, spindle shaped merozoites with an eccentric

Figure 1. Abomasal mucosa. Globidium cyst with compression and atrophy of surrounding glands and widening of the lamina propria. H and E × 110.

Figure 2. Abomasal mucosa. Globidium cyst with destruction of surrounding glands and intense cellular reaction. H and E × 110.

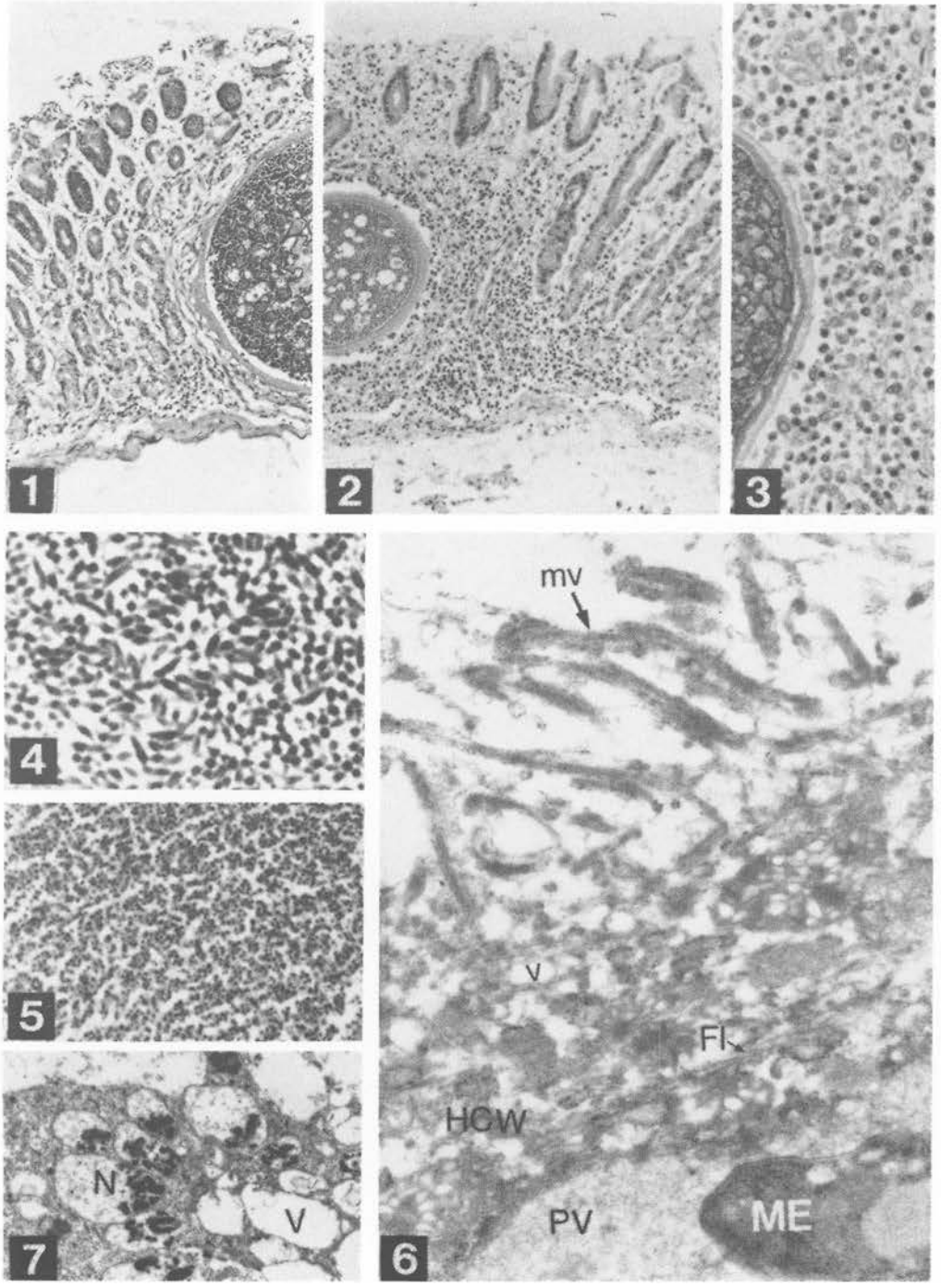
Figure 3. Abomasal mucosa. Globidium cyst with intense mononuclear cell reaction. Note the fimbriae in the outer layer of the cyst wall. H and E × 280.

Figure 4. Elongated merozoites, type I. Toluidine blue × 1,120.

Figure 5. Type II merozoites. H and E × 450.

Figure 6. Part of a host cell representing cyst wall (HCW). Microvilli (mv), vacuole (v), fibrillar elements (FI), parasitophorous vacuole (PV), merozoite (ME). × 13,120.

Figure 7. Immature shizont with many nuclei (N) and vacuoles (V). × 4,500.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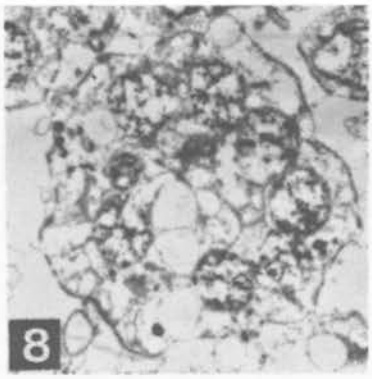
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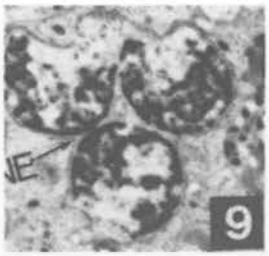
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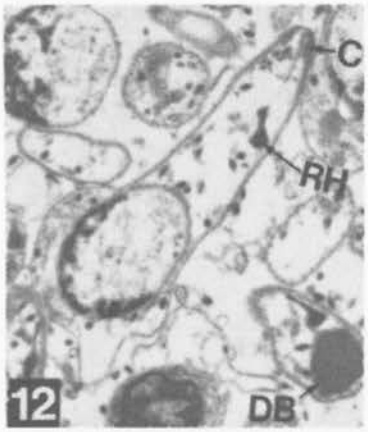
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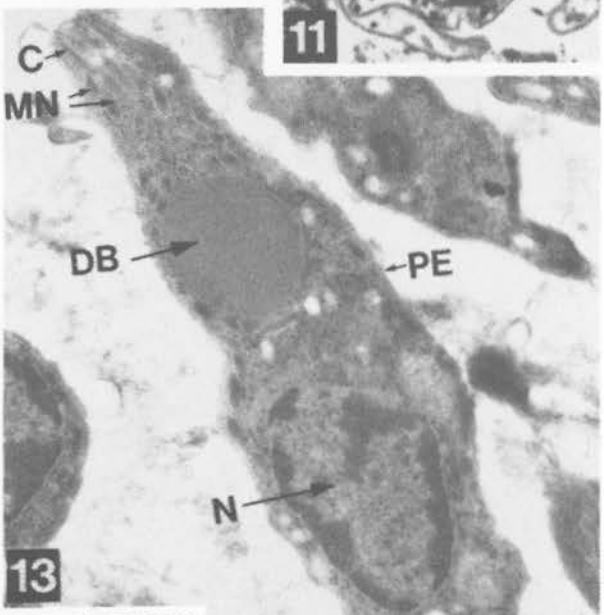
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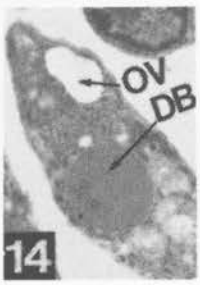
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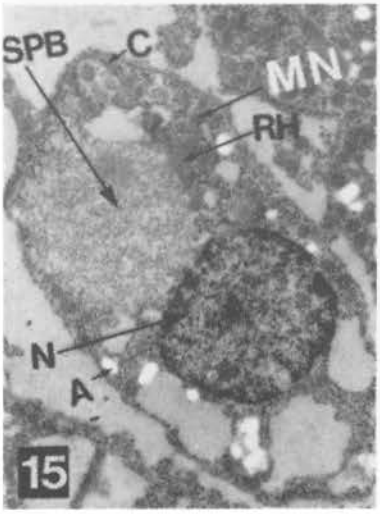
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nucleus (Type I, Fig. 4). The other type of mature cyst contained smaller, more rounded merozoites (Type II, Fig. 5). In some mature cysts, coarse basophilic granular bodies of different sizes were seen scattered among the merozoites.

The tissue around the cysts was compressed and the gastric glands were atrophic (Fig. 1). In most cases there was also a cellular type of reaction around the cyst (Figs. 2 and 3). This reaction was dominated by mononuclear cells of lymphoid and histiocytic type with occasional plasma cells and a few eosinophilic granulocytes. The intensity of this reaction varied from slight to fairly pronounced. It was more pronounced towards the base of the lamina propria than on the other sides of the cyst. This cellular reaction showed no noticeable difference in appearance or intensity between immature and mature cysts. In a few cases the cellular reaction had penetrated the muscularis mucosae and to a smaller degree extended into the submucosa. In some cases the globidium cyst had left the mucosa leaving a crater-shaped erosion. In the edges of this there was a mononuclear cell reaction similar to that around the intact cyst.

Electron microscopic examination

The globidium schizonts were surrounded by an enlarged and transformed host cell representing the cyst wall (Fig. 6). It varied in thickness from 7.1 μm to 15.6 μm. This host cell contained an enormous parasitophorous vacuole which was bounded by a single membrane. The host cell cytoplasm contained several tiny vacuoles and bundles of fibrillar elements. The outer surface of the host cell had numerous microvilli with longitudinal bundles of fibrillar elements.

Some of the immature cysts contained giant schizonts with many nuclei and big vacuoles in the cytoplasm (Fig. 7). In other immature cysts, the cytoplasm was separated into multinucleated cell portions (Fig. 8) which were named blastophores in Eimeria bovis (*Sheffield & Hammond* 1967). The cell portions were unequal in size and irregular in shape and each was limited by a unit membrane. Each cell portion contained a number of nuclei varying from 4 to 60. These nuclei were arranged peripherally. Sometimes two or three nuclei had the same nuclear envelope, indicating that they were undergoing nuclear division (Fig. 9). The cell portions were filled with a well developed endoplas-

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Figure 8. Multinucleated cell portion. × 6,350.

Figure 9. Three nuclei in a cell portion having the same nuclear envelope (NE). × 11,480.

Figure 10. Part of a cell portion showing elevated membrane in front of nucleus forming the conoid (C). × 11,480.

Figure 11. Two developing merozoites before division of the nucleus (N). Pellicle (PE), thick-walled vesicle (DV). × 12,500.

Figure 12. Developing merozoite (finger like bud) with conoid (C), rhoptries (RH) and globular dark body (DB). × 11,700.

Figure 13. Elongated merozoite, type I with nucleus (N), globular dark body (DB), pellicle (PE), micronemes (MN) and conoid (C). × 17,150.

Figure 14. Anterior end of type I merozoite showing oval vacuole (OV) and globular dark body (DB). × 14,700.

Figure 15. Type II merozoite with nucleus (N), spherical body (SPB), conoid (C), micronemes (MN), rhoptries (RH) and amylopectin (A). × 19,950.

mic reticulum and lipid droplets. Later on, the membrane surrounding these cell portions became thick in front of the nuclei and the conoid was formed under it (Fig. 10). This membrane was elevated to form a finger-like bud (Figs. 11 and 12) which is the developing merozoite. This merozoite was covered by a three membrane pellicle characteristic of the motile stages of Apicomplexa (*Chobotar & Scholtyseck* 1982) and contained the anlagen of the anterior portion of the merozoite (conoid, micronemes and rhoptries; Fig. 12).

Usually the nucleus was protruded in the developing merozoite. Sometimes 2 developing merozoites were observed in front of 1 nucleus (Fig. 11) indicating that the last nuclear division would occur. The developing merozoite also contained a thick-walled vesicle (Fig. 11) and sometimes a globular dark body (Fig. 12).

As the development proceeded, the parasite became more elongated and in the last stage of merozoite formation, its outer wall was still attached to the membrane of the cell portions. Finally, the merozoites separated, leaving an irregular shaped residual body.

As mentioned above there were 2 types of merozoites. Both of these develop in the same manner.

Type I merozoite. This merozoite was elongated, spindle in shape and pointed at both ends (Fig. 13). It measured 4.2–5.2 μm × 1.0–1.5 μm (mean 4.8 × 1.3 μm). It was surrounded by a typical coccidian pellicle. The microtubules were 22 in number. The anterior end of the merozoite contained the conoid and usually two rhoptries. A single oval vacuole (0.5 × 0.2 μm) was often found at one end of the merozoite (Fig. 14). The micronemes (20–60 in number) were found mostly in the anterior third of the merozoite and a few in the middle third. A large globular dark body (0.6–0.8 μm) was present in the anterior third. It was surrounded by a unit membrane (Fig. 13).

The nucleus was about 0.8 × 1.6 μm in size and located in the posterior half of the merozoite. The nuclear material appeared as irregular dense clumps mostly at the periphery. The nucleoplasm also contained smaller granules. The mitochondria were located in the middle and posterior third of the merozoite. Amylopectin-like granules were numerous and distributed throughout the cytoplasm of the merozoite.

Type II merozoite. This merozoite (Fig. 15) was oval in shape measuring 3.2–3.6 μm × 1.4–1.9 μm (mean 3.4 × 1.6 μm). The apical complex (conoid, rhoptries) was similar to that in type I. However, the globular dark body was absent, the micronemes were fewer, and a large spherical body (about 0.9 × 1.1 μm) was present at the anterior end. This body contained many granules and an eccentric dense globule. Large irregular vacuoles were usually present posterior to the nucleus. In some merozoites, these vacuoles were located around the nucleus and also in the anterior half of the merozoite. The nucleus was globular (about 1.1 μm) and located in the posterior half of the organism.

Discussion

It has been reported that globidium infection may cause gastrointestinal symptoms with diarrhoea and even death in sheep and goats (*Marsh & Tunicliff* 1941, *Rac & Wilson* 1959, *Mugera & Bitakaramire* 1968, *Tontis et al.* 1977, *Chineme & Njoku* 1978). The morphological picture was then characterized by a severe hemorrhagic gastroenteritis. Our material, however, was collected from apparently healthy and normally slaughtered animals. Consequently, the lesions found here were milder and apparently of no

or very little importance for the health of the individual animals. Some other authors (*Wetzel* 1970, *Jubb et al.* 1985) also found the clinical importance of this parasite in the abomasum to be small or negligible.

Our gross findings of varying amount of solitary, pinhead sized nodules without observable reaction in the intervening parts of the abomasal mucosa were similar to those described earlier (*Alicata* 1930, *Soliman* 1960, *Ferguson & Goldsby* 1961).

Histologically, the main reaction was restricted to the immediate surrounding of the cyst with atrophy of the mucosal glands and mononuclear cell infiltration, which is also in general agreement with earlier reports (*Matta & Pande* 1966, *Mugera & Bitakaramire* 1968). Regarding the intensity of the cellular reaction in relation to the development of the cysts there are, however, some different opinions in the literature. Thus, *Wetzel* (1970) reported an increasing cellular reaction as the globidium cysts were growing to maturity. *Matta & Pande* (1966), on the other hand, found more pronounced cellular reaction around the immature and developing cysts than around the mature cysts.

In our material there was no such difference in degree of pericystic reaction between immature and mature cysts.

Our results regarding the localization of the cysts within the lamina propria of the mucosa and their size and shape are comparable with earlier reports (*Soliman* 1960, *Matta & Pande* 1966).

The basophilic granular bodies observed in some of the mature cysts have been described by *Matta & Pande* (1966) as protoplasmic residual masses. Further studies may clarify their nature.

Our study indicated that the process of merozoite formation in goat globidium was similar to that of Eimeria species (*Chobotar & Scholtyseck* 1982) and of sheep globidium (*Porchet-Hennere* 1977, *Hilali & Scholtyseck* 1979). Type I merozoites reported here were shorter and thicker (4.2–5.2 × 1.0–1.5 μm) than type A merozoites (6.8 × 0.8 μm) described by *Mehlhorn et al.* (1984). Type I had also a vacuole at one end. This was lacking in type A merozoites.

Type II merozoites were mostly similar to type B merozoites described by *Mehlhorn et al.* (1984). However, type II merozoites had a large spherical body at the anterior end, while type B merozoites had instead a small vacuole.

Type I merozoites from our goats has not been described in sheep. Type II merozoites, however were similar to but somewhat smaller than type A merozoites recorded from sheep (*Senaud et al.* 1984). This similarity may suggest a possible common globidian cyst infecting both sheep and goat.

Our knowledge regarding sheep and goat globidia is chiefly based on morphological descriptions of the cysts and their contents (giant schizonts).

Two different merozoites were reported in our study and two other merozoites were reported by *Mehlhorn et al.* (1984). Whether these represent developmental phases of the same merozoite or different globidian parasites is still unknown.

The life cycle and taxonomic status of globidium is doubtful. It is considered to be a merogenious stage of one or more of the known ovine or caprine Eimeria spp (*Levine* 1973).

In a previous study on the same goats (*Abdurahman* 1985) 9 Eimeria spp were found in globidia positive animals. However, none of these species was statistically associable with globidium. Equally, the lack of association could not be conclusive evidence of globidia not being Eimeria. *Sénaud et al.* (1984) indicated that some merozoites of

sheep globidia did not develop in tissue culture of sheep, goat or dog cells.

Further studies are needed to try to inoculate the different merozoite forms of sheep and goat globidias in cultured cells of sheep, goat and different carnivors. This may clarify the life cycle of globidian parasites.

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Sammanfattning

Globidios hos get

Hos synbarligen friska getter från Somalia påvisades knappnålshuvudstora knutor i löpmagens mucosa. Knutorna utgjordes av inkapslade cystor innehållande mogna eller omogna schizonter. Omkring cystorna sågs ofta atrofi av körtlar och lymfohistiocytär cellreaktion. Ultrastructuren av omogna och mogna cystor beskrivs i detalj. Mogna cystor innehöll långsträckta, spolformade merozoiter (typ I) eller kortare, ovala merozoiter (typ II). I en del mogna cystor fanns också basofila granulära kroppar bland merozoiterna. Typ I och II merozoiter skiljde sig morfologiskt från de som tidigare beskrivits hos get.

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