SHORT COMMUNICATION

Encephalitozoon-Like Microsporidia in the Ticks *Amblyomma cajennense* and *Anocentor nitens* (Acari: Ixodidae)

MÚCIO FLÁVIO BARBOSA RIBEIRO AND ANTÔNIO MARCOS GUIMARÃES[1]

Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 486, CEP 31.270-910, Belo Horizonte, Minas Gerais, Brasil

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ABSTRACT A *Encephalitozoon*-like microsporidia was found in epithelial cells of the midgut and the salivary glands of *Amblyomma cajennense* (F.) and *Anocentor nitens* (Neumann) that had fed on rabbits. Ultrastructural studies demonstrated that all stages of the life cycle of the parasite occur in parasitophorous vacuoles and contain only 1 nucleus. The sporonts detach from the limiting membrane of the vacuole and divide by binary fission to produce the sporoblasts, each presenting a thickened electron-dense wall, and a primordium of a polar filament. Each spore contained a single nucleus, an electron-dense and rough exospore, an electron-lucent and thick endospore, and 5 coils of the polar tubule.

KEY WORDS *Amblyomma cajennense, Anocentor nitens,* Microsporidia, *Encephalitozoon*-like

MICROSPORIDIANS ARE INTRACELLULAR protozoans of the phylum Microspora that infect invertebrates, fish, and mammals (Canning 1977). More recently, cases have been reported for humans, and microsporidia have attracted attention as examples of opportunistic pathogens (Canning and Hollister 1992, Bryan and Weber 1993). The incidence of human microsporidiosis has increased in association with human immunodeficiency virus (HIV) infection (Didier et al. 1991, Schwartz et al. 1996).

More than 700 species of microsporidians have been reported, belonging to 118 genera (Sprague et al. 1992). They have a high reproductive capacity, and are characterized by their production of spores that contain a coiled polar filament and the sporoplasm which contains 1 or 2 nuclei (Cali 1991). The sources of infection and the mechanisms of transmission of these organisms in humans are still uncertain. The ingestion of spores, a resistant form of the parasite, is the most probable source of infection. The transmission of invertebrate microsporidia to mammals is normally considered impossible. Microsporidians of the genus *Nosema* have been described in *Ixodes ricinus* (L.) (Weiser and Rehácek 1975), *Dermacentor reticulatus* (F.) and *Ornithodoros parkeri* (Cooley) (Krinsky 1977). Attempts to infect Swiss mice by tick feeding or by injection of infected tick suspensions have been unsuccessful. However, the experimental inoculation of spores of *Nosema algerae* (Vavra and Undeen), a mosquito parasite in athymic mice, has been successful in causing infection. This finding suggests that the possibility of invertebrate microsporidia as a source of human microsporidiosis should now be taken seriously (Trammer et al. 1997).

The aim of this research is to describe the occurrence of microsporidians in the midgut epithelial cells and salivary glands of the ixodid ticks *Amblyomma cajennense* (F.) and Anocentor nitens (Neumann).

Materials and Methods

Engorged female ticks of *A. nitens* and *A. cajennense,* collected from naturally infested horses and cattle, were washed, blotted dry, placed in petri dishes, and maintained in an incubator at 27°C and 90% RH. The 1st eggs were collected and packed in glass tubes and maintained in the incubator until eclosion. Approximately 1,000 *A nitens* larvae, obtained 14–21 d after eclosion, were placed on a foal free from hemoparasites. On reaching the nymphal stage, they were collected and maintained for ecdysis. Because *A. cajennense* is a 3-host tick, the larval and nymphal stage were fed on rabbits and equine hosts. Nymphs were collected and maintained under the same conditions as those described above. After molting, the adult ticks of both species were fed on rabbits. They were manually detached 2–4 d after attachment.

Preparation of Tick Tissues for Microscopy. Midgut and salivary tissue of each species were collected from groups of 10 female adult ticks. Tissue samples were immediately placed in cold 2% glutaraldehyde in 0.25 M cacodylate buffer, and postfixed in 2% osmium tetroxide and 0.25 M cacodylate buffer. The fixed tissue was washed several times in cacodylate buffer, dehy-

In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals," as promulgated by Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

[1] Escola de Veterinária da Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

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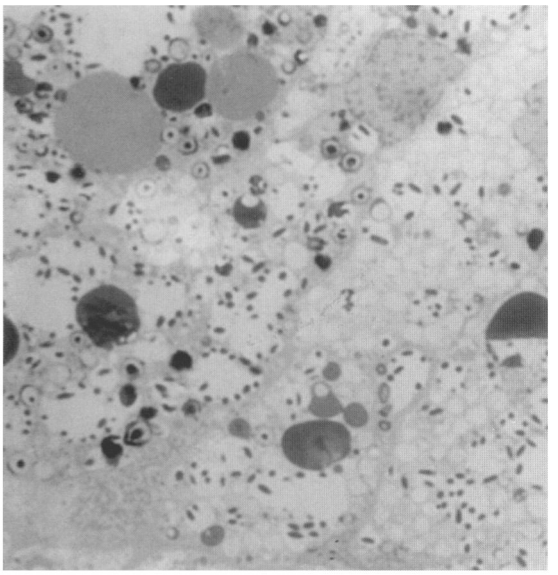

Fig. 1. Photomicrograph of *Encephalitozoon*-like organism in midgut epithelial cells of *A. nitens*. The cytoplasm was occupied by numerous development stages and spores. Stained 1-μm plastic section (1,600×).

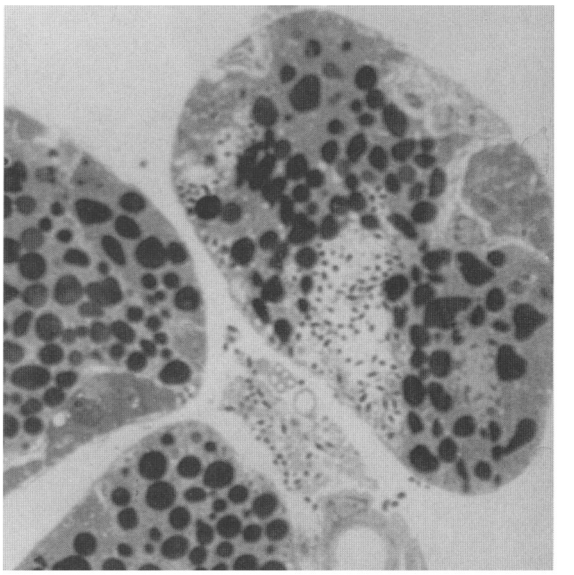

Fig. 2. Photomicrograph of *Encephalitozoon*-like organism in the e cell of salivary gland of the tick. Stained 1-μm plastic section (1,600×).

drated through a 50–100% ethanol series, and embedded in Poly/Bed 812 (Polysciences, Warrington, PA). Sections 1 μm thick were stained with toluidine blue at 60°C and examined by light microscopy. Ultrathin sections (60–90 nm), cut with a diamond knife, were collected on 300-mesh cooper grids, stained with uranyl acetate and lead citrate, and examined using a transmission electron microscope (Zeiss-M 10).

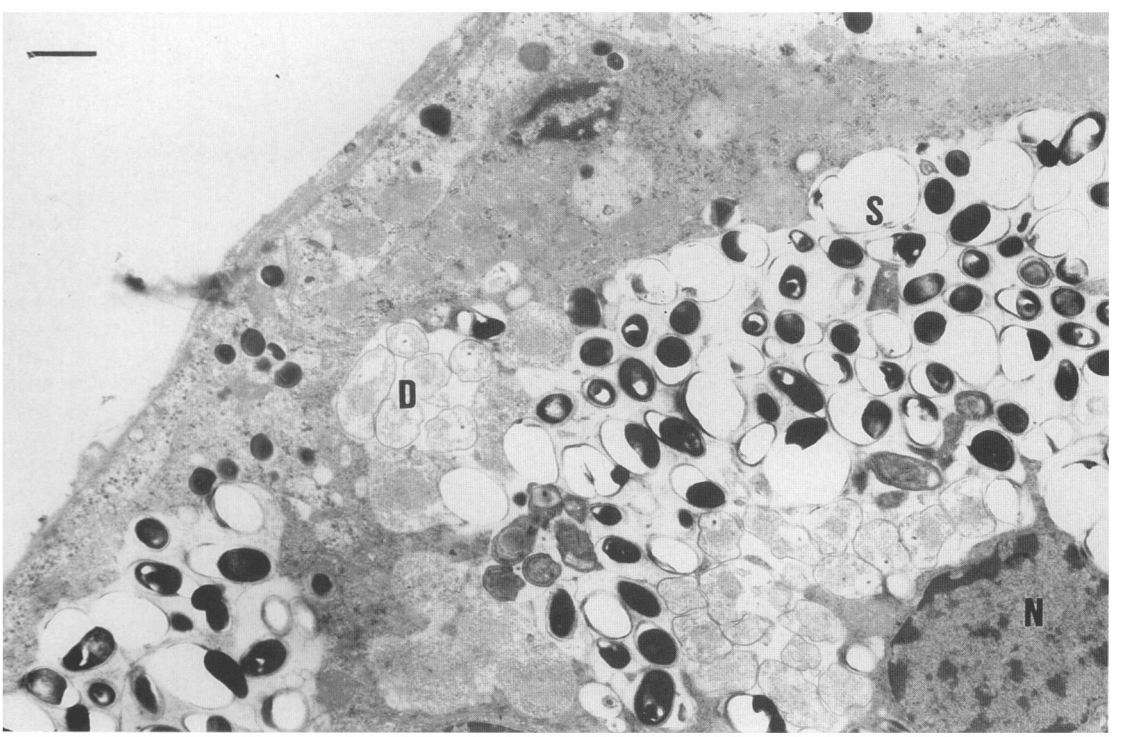

Fig. 3. Electron-micrographs of development stages and spore of *Encephalitozoon*-like organism infecting the midgut epithelial cells of *A. nitens*. Bar = 1.4 μm. D, development stage; N, host cell nucleus; S, spore (6,400×).

Results

Light Microscopy. Parasites were found in the midgut epithelial cells and in the acinus of the salivary glands of ticks 4 d after feeding on rabbits. At this stage, 50% (5 of 10) of the *A. nitens* and 30% (3 of 10) of the *A. cajennense* females were infected. The main site of parasite development was in the midgut epithelial cells. The accumulation of parasites in the cytoplasm damaged the host cells (Fig. 1).

In the salivary glands, the spores of the parasite were observed in type III acini within the e cells (Binnington 1978). After growing, they split into numerous vacuoles (Fig. 2).

Transmission Electron Microscopy. Parasites observed in the midgut epithelial cells and in the salivary glands of the ticks presented different morphological stages, suggesting a sequential phase of development (Fig. 3). Meronts were observed attached to the membrane of parasitophorous vacuoles, presenting round, oval, and elongate shapes that ranged in size from 1.4–2.7 μm in length to 2.1–7.0 μm in width. Each contained a prominent nucleus. We observed ribosomes, small endoplasmic vesicles, and remarkably rough endoplasmic reticulum in the cytoplasm (Fig. 4B).

When sporogony began, meronts changed into sporonts, and the cisternae of the rough endoplasmic reticulum were arranged in well-defined concentric layers around the nucleus. There was a deposition of granules on the surface of the parasite, making the walls thicker and more electron-dense. The sporonts detached from the limiting membrane, and had oval or elongate shapes. They multiplied by binary fission, producing the sporoblast, that contained a single nucleus (Fig. 4A). This stage had a rough endoplasmic reticulum that surrounded the nucleus and the primordium of the polar filament. In transverse cuts of the sporoblasts, in an advanced state of development, we observed 4–6 coils of the polar tubule, some of them presenting extrusion of their polar tubules (Fig. 4B, arrow head). The sporoblasts changed into spores with a ovoid shape that measured from 1.2 to 1.7 μm in length to 0.4 to 0.8 μm in width.

The spores had a trilayered wall that consisted of an outer, rough, electron-dense exospore, a thicker, electron-lucent endospore, and a fine electron-dense plasmalemma (Fig. 5 A–C). The spore cytoplasm contained a single nucleus, a posterior vacuole, and a polar tubule with 4–8 coils (mean of 5).

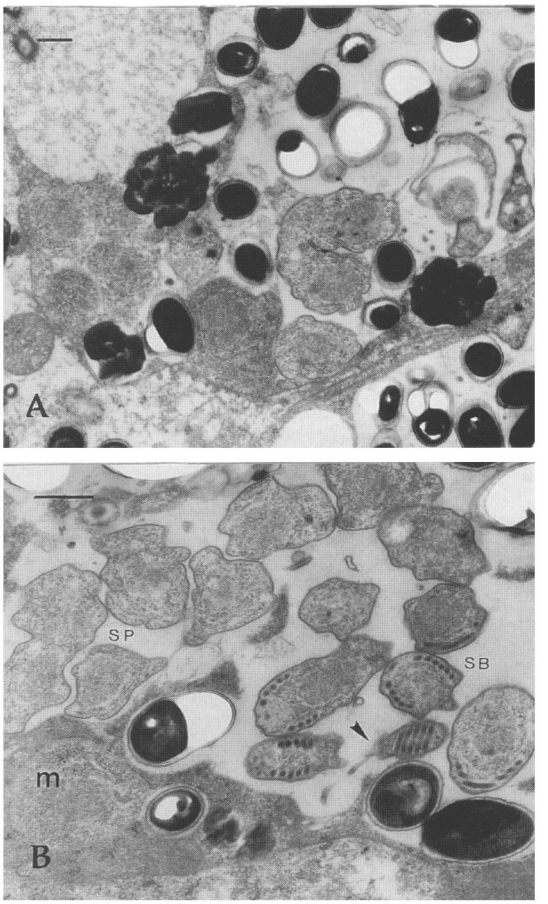

Fig. 4. Electron-micrographs of *Encephalitozoon*-like organism within cytoplasm of midgut epithelial cells of *A. nitens*. Bar = 0.60 μm. (A) Sporogamy; a sporont dividing into 2 sporoblasts. Note that walls are thicker and more electron-dense. Some mature spores also appear in the vacuole. (12,840×). (B) Development stages of parasite meront attached to membrane of parasitophoro vacuole (m); sporonts oval and elongate shape (SP) and sporoblast (SB) with polar tubules coiled. Note a sporoblast with extrusion of its polar tubule (arrow head) (20,000×).

Discussion

This is the first description of the ultrastructure of a microsporidian resembling the genus *Encephalitozoon* identified in ticks The parasite observed in the midgut epithelial cells and in the acinus of the salivary glands of *A. cajennense* and *A. nitens* showed, in 1 of its biological stages, the formation of spores that contained a coiled polar filament and sporoplasm with a single nucleus. This is a distinguishing feature of the phylum Microspora (Cali 1991). The identification of genera is based on ultrastructural studies to verify the size of the spore, the nuclear arrangement, and the parasite development in the host cell (Furuya et al. 1995). Ultrastructural observations of the stage of microspore sporogony in the ticks included in this study confirmed that all of the parasitic stages that developed inside the parasitophorous vacuoles were monokaryotic, and produced uninucleated spores that showed 4–8 coils of the polar tubule. Based on these findings, it was classified as probably belonging to the genus *Encephalitozoon* (Sprague et al. 1992).

Cali (1991) reported that the number of coils in the polar filament of *Encephalitozoon* spp. varies, and that it is closely related to the parasite species. Currently, 3 species are recognized for the genus—*E. cuniculi* (Levaditi, Nicolau, & Schoen), which presents 4–5 coils of the polar tubule (Canning 1977); *E. hellem*

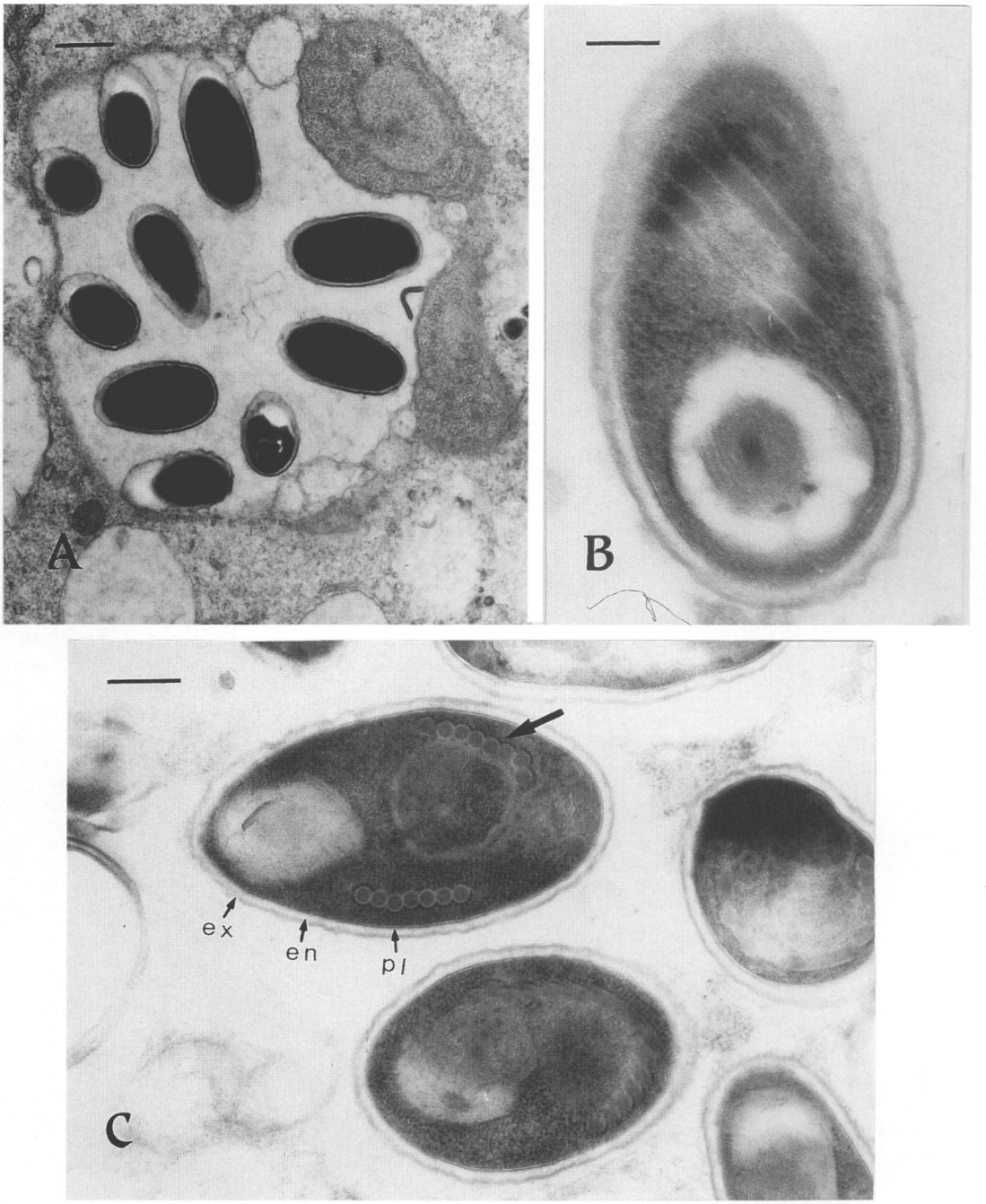

Fig. 5. Electron-micrographs of mature spore of *Encephalitozoon*-like organism. (A) Parasitophorous vacuole content mature spores and development stages of parasite. Bar = 0.50 μm. (B) Transverse cut of mature spore with a coiled polar tubule. Bar = 0.15 μm. (C) mature spore with electron-dense exospore (ex), thick electron-transparent endospore (en) thin plasmalemma (pl), coils of the polar tubule (arrow). Bar = 0.30 μm.

(Didier et al.), which presents 4–9 coils (Didier et al. 1991); and an unclassified species that has 6 coils of the polar tubule (Desser et al. 1992). The microsporidian found in the ticks had 4–8 coils in the polar tubule, which made it difficult to identify based only on this morphological character. Serologic tests should be conducted to identify correctly the species involved in the infection of these ixodids (Didier et al. 1991).

The source of the infection observed in the ticks is unknown because microsporidia were collected from horses and cattle and subsequently maintained on rabbits. However, the fact that midgut epithelial cells and

the salivary glands of the ticks were heavily infected only on day 4 after feeding on rabbits, suggested that the rabbit blood meal was the source of infection. Encephalitozoonosis is normally asymptomatic, chronic, and rarely fatal in immunocompetent rabbits (Shadduck and Pakes 1971). Spores of *E. cuniculi* inoculated directly into the brain of a rabbit caused encephalitis and interstitial nephritis (Shadduck et al. 1979), showing the rapid spread of spores as soon as they reached the blood. Ticks are highly sensitive to the parasite, and consequently multiplication is rapid, not only in the midgut epithelial cells but also in the salivary glands. The biological cycle of *Encephalitozoon* inside these cells occurs rapidly; the sporogony stage began within 4 d after feeding. These observations are similar to those found for the *E. cuniculi* cycle in cell cultures by Pakes et al. (1975).

The fact that *Encephalitozoon*-like microsporidia spores were found in the salivary glands of the ticks, most particularly in *A. cajennense* which is a 3-host tick, suggests that they may play an important role in the transmission of the parasite in nature, including to humans. During the larval stage, *A. cajennense* feeds on wild mammals such as rabbits, and the infected ticks could subsequently transmit the parasite to other mammals during their next blood feeding. This tick is recognized as one of the vectors of Rocky Mountain spotted fever, which is caused by *Rickettsia rickettsii* in Brazil.

Acknowledgments

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