

Ultrastructure of *Babesia equi* trophozoites isolated in Minas Gerais, Brazil¹

Antônio M. Guimarães², José D. Lima³ and Múcio F. B. Ribeiro³

ABSTRACT.- Guimarães A.M., Lima J.D. & Ribeiro M.F.B. 2003. Ultrastructure of *Babesia equi* trophozoites isolated in Minas Gerais, Brazil. *Pesquisa Veterinária Brasileira* 23(3):101-104. Departamento de Medicina Veterinária, Universidade Federal de Lavras, Cx. Postal 37, Lavras, MG 37200-000, Brazil. E-mail: amg@ufla.br

A transmission electron microscope study was carried out on *Babesia equi* obtained from a splenectomized horse, from the municipality of Santa Luzia, Minas Gerais, Brazil. The isolate was inoculated into two splenectomized foals (1.05×10^{10} parasitized erythrocytes by *B. equi*). Trophozoites have a single membrane in direct contact with the cytoplasm of the red blood cells, a prominent nucleus, well-developed rough and smooth endoplasmic reticulum, numerous free ribosomes and small food vacuoles. *B. equi* trophozoites have a cytostome and a long tubular feeding structure in direct contact with the blood plasma.

INDEX TERMS: *Babesia equi*, ultrastructure, trophozoites, feeding mechanism.

RESUMO.- [Ultra-estrutura de trofozoítos de *Babesia equi* isolados em Minas Gerais, Brasil.] Neste estudo de microscopia eletrônica de transmissão utilizou-se um isolado de *Babesia equi* obtido a partir de um equino esplenectomizado, oriundo do município de Santa Luzia, Minas Gerais, Brasil. O isolado foi inoculado em dois potros esplenectomizados ($1,05 \times 10^{10}$ eritrócitos parasitados com *B. equi*). Os trofozoítos apresentaram uma membrana simples em contato direto com o citoplasma das hemácias, núcleo proeminente, retículo endoplasmático liso e rugoso bem desenvolvidos, numerosos ribossomos livres e pequenos vacúolos alimentares. Em trofozoítos de *B. equi* foi observado citostoma e uma longa estrutura tubular de alimentação em contato direto com o plasma sanguíneo.

TERMOS DE INDEXAÇÃO: *Babesia equi*, ultra-estrutura, trofozoítos, mecanismos de alimentação.

INTRODUCTION

Equine babesiosis is an infectious disease of horses and other equids caused by the protozoan hemoparasites *Babesia equi* and *Babesia caballi* (De Waal 1992). *B. equi* infects 90% of the

world equine population (Schein 1988), and is transmitted naturally by ticks of the genera *Hyalomma*, *Dermacentor* and *Rhipicephalus* (Friedhoff 1988), and experimentally by *Boophilus microplus* (Knowles et al. 1992, Guimarães et al. 1998a,b). *B. equi* was first isolated in Brazil by Ribeiro & Lima (1989), from naturally infected horses to produce an antigen for serological purposes. Although the disease is probably endemic in Brazil, only a small number of studies were published, all of them based on clinical and serological surveys (Guimarães et al. 1997, Heuchert et al. 1999, Kerber et al. 1999, Ribeiro et al. 1999).

Intracellular parasites depend on the host cell to obtain the majority of their nutrients during development. In *Plasmodium* spp the parasites ingest hemoglobin through a cytostome (Aikawa et al. 1966) and digestion is through proteolytic enzymes in digestive vesicles (Slomianny et al. 1983). Electron-dense hemozoin pigments in food vacuoles comprise the final product of hemoglobin digestion (Aikawa 1971). The feeding mechanism of *Babesia* spp remains incompletely described, and the formation of food vacuoles has been the subject of some controversy (Langreth & Trager 1976, Rudzinska 1976). Electron microscope studies on *B. equi* trophozoites have demonstrated a cytostome and a tubular food vacuole (Simpson et al. 1967, Frerichs & Holbrook 1974, Simpson & Neal 1980) involved in the formation of food vacuoles, similar to the observed in some species of *Theileria* (Higuchi et al. 1984, Conrad et al. 1985, Fawcett et al. 1987).

This paper describes a transmission electron microscope (TEM) study of *Babesia equi* trophozoites with emphasis on the structures involved in nutrient intake.

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²Departamento de Medicina Veterinária, Universidade Federal de Lavras, Cx. Postal 37, Lavras, MG 37200-000, Brasil. E-mail: amg@ufla.br

³Departamento de Parasitologia, ICB, Universidade Federal de Minas Gerais, Cx. Postal 486, Belo Horizonte, MG 31270-901, Brasil.

MATERIALS AND METHODS

The strain of *Babesia equi* (BE/MG) used in this study was isolated following splenectomy of a naturally infected 17-month old crossbred foal from Santa Luzia town, State of Minas Gerais, Brazil. The foal had been medicated previously with diminazene diaceturate; two applications of 6 mg/kg, at intervals of 24 hours. This was to eliminate possible infection with *Babesia caballi*. The foal was kept free of ticks by spraying weekly with deltamethrin. It developed relapse of *B. equi* 22 days following splenectomy. Thirteen days later, smears of peripheral blood stained with Giemsa demonstrated that 44% of the red cells were infected. Subsequently, blood was collected by jugular venipuncture using EDTA as anticoagulant and preserved in liquid nitrogen using dimethylsulphoxide.

Two 12 and 15-month old crossbred, splenectomized foals were used for experimental infection. They had been maintained free of ticks since birth by weekly spraying with deltamethrin. They were inoculated intravenously with 1.05×10^{10} *B. equi* (BE/MG) infected erythrocytes. After achieving 40% of the red cells infected, peripheral blood was collected as described above.

After centrifugation at 1,400g for 5 minutes, plasma and leucocyte coat were removed. Erythrocytes were fixed by the addition of 500 μ l of sediment to 5 ml of a fixative containing 2.5% glutaraldehyde in sodium cacodylate buffer 0.1M, pH 7.2, for 24 hours at 4°C. Fixed cells were then centrifuged at 1,400g for 5 minutes, washed in cacodylate buffer and post-fixed in 2% osmium tetroxide in 0.1M

cacodylate buffered, for 2 hours at 4°C and subsequently set in 2% agar. After three washes in PBS with 17.8% sucrose, the samples were stained "en bloc" with uranyl acetate at 0.5% (w/v) in aqueous solution with 13.3% sucrose, at 4°C for 14 hours. The samples were then washed three times in aqueous solution with 13.3% sucrose, dehydrated in increasing concentrations of ethyl alcohol, and embedded in epon-araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined using a transmission electron microscope Zeiss EM 10A.

RESULTS

Trophozoites of *Babesia equi* were round shaped with 1.3 μ m diameter, covered by single membrane in direct contact with the cytoplasm of the erythrocyte (Fig. 1). There was observed a prominent nucleus (0.8 μ m in diameter) with a double membrane, well-developed rough and smooth endoplasmic reticulum, vacuoles and numerous free ribosomes (Fig. 2).

Trophozoites presented a single cytostome composed of a single electron-dense concentric ring (terminal fringes), with an internal diameter of 0.1 μ m and located on the periphery of the parasite (Fig. 3). The material inside the cavity of the cytostome was of the same density as the cytoplasm of the erythrocyte. Small intracytoplasmic food vacuoles were observed next to the cytostome. Food vacuoles were

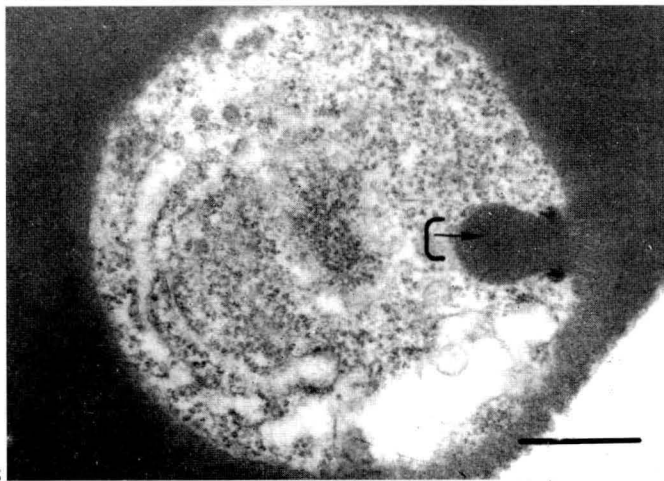
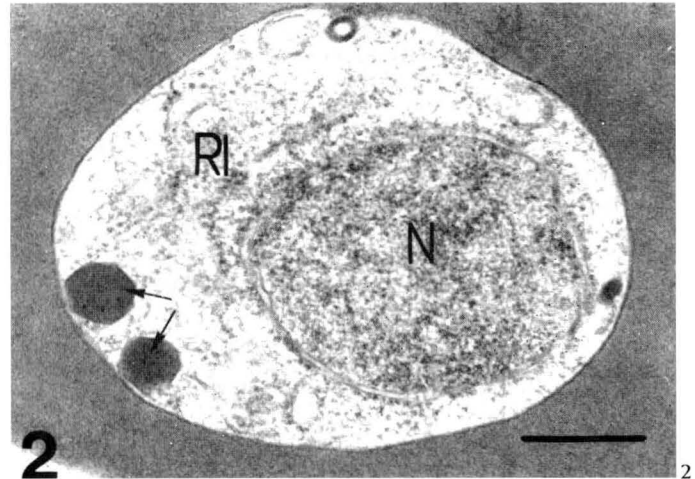
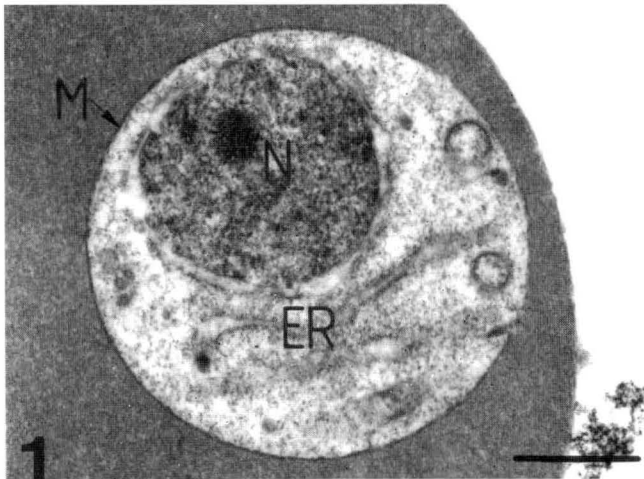


Fig. 1. Electron micrograph of *Babesia equi*. Bar: 0.4 μ m. The trophozoite was bounded a single unit membrane (M). N = Nucleus, ER = Endoplasmic reticulum.

Fig. 2. Electron micrograph of *Babesia equi*. Bar: 0.3 μ m. The trophozoite within equine red blood cell. Note nucleus (N), two food vacuoles (arrows) and ribosomes (RI) distributed in the parasite cytoplasm.

Fig. 3. Electron micrograph of *Babesia equi*. Bar: 0.4 μ m. The trophozoite showing a cytostome (C).

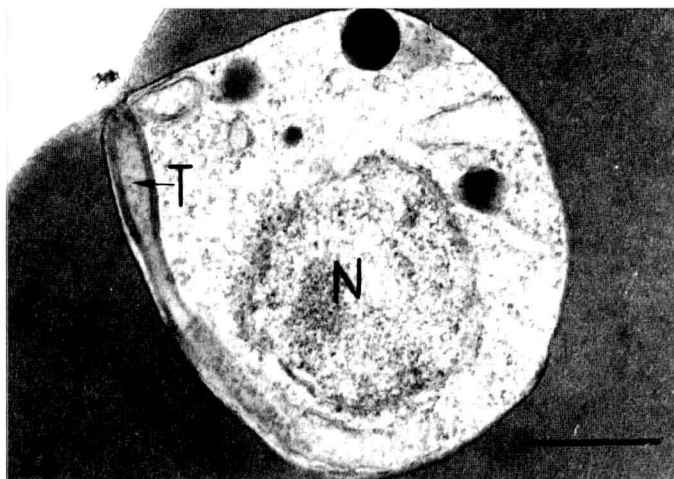


Fig. 4. Electron micrograph of *Babesia equi*. Bar: 0.3 μm . The trophozoite of *B. equi* showing a tubular food vacuole (T). N = Nucleus.

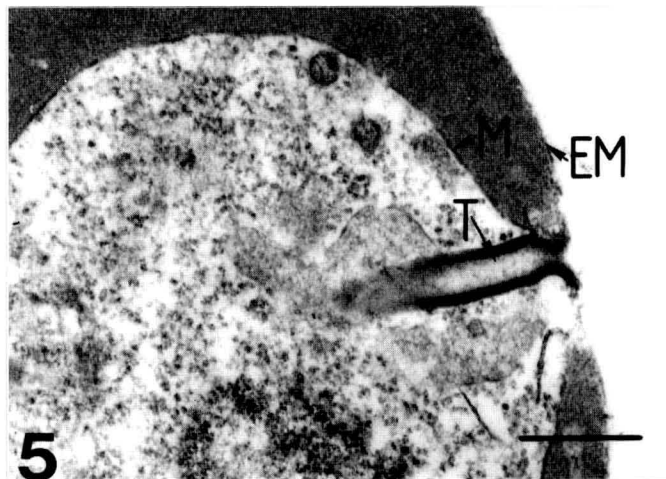


Fig. 5. Electron micrograph of trophozoite of *Babesia equi*. Bar: 0.3 μm . The tubular food vacuole (T) is formed by the concomitant invagination of the plasma membranes of erythrocyte (EM) and the parasite (M).

eventually observed, surrounded by a single cytoplasmic membrane and containing material of similar density to hemoglobin.

Trophozoites located very close to the erythrocyte membrane presented a tubular feeding structure, which emerged from the interior of the parasite and extended to the blood plasma (Fig. 4). This tubular feeding structure was formed by the concomitant invagination of the plasma membranes of the erythrocyte and the parasite (Fig. 5). These membranes were in intimate contact throughout the invagination, and were separated by a central lumen measuring 60 ηm , containing a small quantity of ingested material. The invaginations of the plasma membrane of the trophozoites enclosed small quantities of the cell hemoglobin. The ingested material appeared to be continuous with and had the same intensity of color as the rest of the host erythrocyte cytoplasm.

DISCUSSION

The results corroborate and detail the previous descriptions of the ultrastructure of *Babesia equi* (Simpson et al. 1967, Simpson 1970, Frerichs & Holbrook 1974, Simpson & Neal 1980). The electronmicrographs of this paper show clearly that *B. equi* trophozoite is surrounded by a single membrane and is not contained in a parasitophorous vacuole. It is apparent that its presence does not provoke evident changes in the cytoplasm of the host cell. All the stages have a prominent nucleus, well-developed rough and smooth endoplasmic reticulum, and many free ribosomes. Mitochondrial organelles were not observed in the intra-erythrocytic stages of *B. equi*, and typical mitochondria have not been recorded in any previous studies (Simpson et al. 1967, Simpson 1970, Frerichs & Holbrook 1974, Simpson & Neal 1980).

A singular aspect of the morphology of the *B. equi* trophozoites is the presence of a cytostome and the tubular

feeding structure involved in the feeding mechanism (Frerichs & Holbrook 1974). The cytostome was first described in *Plasmodium* spp (Aikawa et al. 1966), and a cytostome together with a tubular food vacuole has been described in some species of *Theileria* (Higuchi et al. 1984, Conrad et al. 1986, Fawcett et al. 1987). In this study, the analysis of electronmicrographs allowed discussion of only certain aspects of the feeding mechanism of *B. equi*. These included the detection of pinocytosis, and the formation of food vacuoles, along with the presence of a cytostome and tubular feeding structure. Food vacuoles were observed in the *B. equi* trophozoites which had the same density as the erythrocyte cytoplasm, lacked a limiting membrane, and showed continuity between the vacuole contents and the cytoplasm of the host cell. A similar situation has been described in *Babesia rodhaini* (Rudzinska & Trager 1960, 1962), *Babesia canis* and *Babesia caballi* (Simpson et al. 1963), *Babesia felis* (Denning & Hebel 1969) and *Babesia microti* (Shortt & Blackie 1965, Mcmillan & Brocklesby 1971). Rudzinska (1976) demonstrated that these food vacuoles are not formed through phagocytosis and called them pseudo-food vacuoles. According to this author, the reason for this phenomenon is unknown, but they probably serve to increase the surface area of the parasite which is in direct contact with the host cytoplasm, and facilitate as such the ingestion of nutrients.

In this study, some trophozoites were found to have a well-developed cytostome and food vacuole. The material in the cytostome cavity was of the same density as that observed for the erythrocyte cytoplasm. Some *B. equi* trophozoites were eventually observed with food vacuoles surrounded by a single membrane, without continuity between the vacuole contents and cytoplasm of the erythrocyte. This type of vacuole did not, therefore, arise from the surface of the parasite through pinocytosis, but probably through expansion of the base of the cytostome and its subsequent separation by constriction.

In this case, it can be considered a true food vacuole formed by cytotosomal phagocytosis of the hemoglobin. The fact that a continuity between the cytostome and the food vacuole was not observed in this study may be attributed in part to the transitory nature of the connection between the two structures. In contrast to other *Babesia* species, it is possible that the trophozoites of *B. equi* are capable of ingesting hemoglobin through both cytostomes and pinocytosis. Intraerythrocytic stages of *Theileria* spp with a cytostome and the formation of food vacuoles were described in the electron microscope studies of Conrad et al. (1986) and Fawcett et al. (1987).

B. equi is able to form a tubular food vacuole from the extensive invagination of the plasma membranes of the erythrocyte and the trophozoite, when the parasite is located very close to the periphery of the host cell (Simpson et al. 1967, Simpson & Neal 1980). A similar structure has been described in species of *Theileria* (Conrad et al. 1986, Fawcett et al. 1987). The tubular structure extends from the interior of the trophozoite to the periphery of the erythrocyte, where its lumen frequently maintains direct contact with the blood plasma, probably allowing to ingest nutrients during periods of rapid growth and development of the parasite (Frerichs & Holbrook 1974). Higuchi et al. (1984) observed similar tubules in *Theileria sergenti* along with "excreta-like" material outside the erythrocyte in the region where the tubule was in contact with the parasite. Higuchi et al. (1984) argued that the parasite probably excretes actively from the tubular structure. Controversy persists, however, with regard to the role of this structure (ingestion or excretion), and further studies are required to determine its true function.

While taking into account the inherent limitations of the method, based on an analysis of the electronmicrographs, it is possible to conclude that *Babesia equi* can form true food vacuoles and use the cytostome, the tubular feeding structure and pinocytosis as feeding mechanisms.

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