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THE FINE STRUCTURE OF DEVELOPMENTAL STAGES OF *BABESIA CABALLI* IN THE SALIVARY GLANDS OF *HYALOMMA TRUNCATUM*

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ABSTRACT

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The development of *Babesia caballi* in the salivary glands of *Hyalomma truncatum* was studied at the electron microscopic level. Kinetes were first observed in the salivary glands of ticks on Day 2 of tick feeding and on each subsequent day of feeding until engorgement on Day 8. Sporogony appeared to involve the formation of cytomeres. After continued nuclear division, sporozoites formed when individual rounded nuclei were incorporated into portions of cytoplasm. Sporozoites were first observed on Day 4 of tick feeding and contained typical *Babesia* spp. organelles with a polar ring and up to 4 rhoptries, spherical bodies, a nucleus, mitochondria, endoplasmic reticulum and micronemes. The infection rate in the ticks was approximately 80 %.

INTRODUCTION

Babesia caballi is one of the 2 species which cause equine babesiosis in the Republic of South Africa. Nuttall & Strickland (1912) identified both *Babesia equi* and *B. caballi* as causative agents of the disease and indicated that each produced a different clinical syndrome. Although Neitz (1956) listed 9 species of ixodid ticks which may serve as vectors of *B. caballi*, there is a paucity of information regarding both the parasite and its vectors.

The efficiency of the different tick species in the transmission of *B. caballi* may vary considerably. Holbrook, Anthony & Johnson (1968) reported that, although *Dermacentor nitens* was shown to be a vector, only a small percentage of ticks became infected. Neitz (1956) indicated that some vectors of *B. caballi* could remain infective for at least 2 generations without feeding on an infected animal, while others maintained an infection for only 2 successive life cycle stages of the same generation.

Hyalomma truncatum occurs on both wild and domestic animals and has a wide distribution in South Africa (Howell, Walker & Nevill, 1978). Recent laboratory studies have established *H. truncatum* as a vector of *B. caballi* and indications are that it is an efficient one (De Waal, 1989).

There have been a number of light microscopic studies of *B. caballi* in its respective tick vectors (Tsaprun, 1941, 1952, 1954; Shepelev, 1942; Abramov, 1955, as cited by Holbrook *et al.*, 1968). Mehlhorn & Schein (1984) have reviewed a number of electron microscopic studies on several other *Babesia* spp. in their vectors which show differences in developmental patterns. The present study reports on the observations made of *B. caballi* in the salivary glands of *H. truncatum*.

MATERIALS AND METHODS

Infection of ticks

One hundred adult *H. truncatum* maintained in the laboratory for several generations were fed on the shoulder region of a horse (De Waal & Potgieter, 1987) which received 250 m*l* of blood intravenously from a horse with a natural infection of *B. caballi*. At the time of tick feeding the parasitaemia was so low that it could only be detected in thick blood films. The engorged adults were placed in an acaridarium to lay eggs and were maintained at 26 °C and 85 % relative humidity, after the methods of Neitz, Boughton & Walters (1971). Infected female ticks were identified by finding kinetes in Giemsa-stained haemolymph smears obtained during egg laying. The ensuing larval and nymphal stages of infected ticks were fed on rabbits and maintained as described above.

Transmission of the parasite

After moulting, approximately 100 ticks of the subsequent adult generation were fed on a susceptible horse. Five to 10 ticks were collected daily, beginning with the day of infestation (Day 0) until engorgement on Day 8 post-infestation and prepared for electron microscopy.

Electron microscopic techniques

Haemolymph samples were collected from the haemocoel of engorged female ticks with a microlitre syringe[1] and released into cold 2 % glutaraldehyde in 0,2 M sodium cacodylate buffer with 0,5 % sucrose (pH 7,4) and centrifuged at 15 000 *g* for 3 min. After spinning, fresh glutaraldehyde was added to the pellet.

After collection, each tick was submerged into a similar solution of cold 2 % glutaraldehyde and its dorsal exoskeleton was removed. The salivary glands were dissected out and transferred to fresh 2 % glutaraldehyde. The glands and haemolymph pellet were post-fixed in 2 % osmium tetroxide in 0,2 M sodium cacodylate buffer, dehydrated in a graded ethanol series, passed through propylene oxide as a transitional solvent and embedded in Polarbed[2].

Thick sections of 1 µm were cut and stained with Mallory's stain after Richardson, Jarret & Finke (1960) for light microscopic viewing.

Ultrathin sections (silver reflective) were cut on a Reichert-Jung ultramicrotome and stained with uranyl acetate and lead citrate (Venable & Coggeshall, 1978). Sections were viewed with a JEOL JEM 1200EX electron microscope.

RESULTS

Infection of ticks and subsequent transmission of the parasite

The horse that received the inoculation of *B. caballi* infected blood underwent a mild clinical reaction and spontaneously recovered. The prepatent period was 11 days post-inoculation and a parasitaemia could only be determined in thick blood films during tick feeding. Positive haemolymph smears

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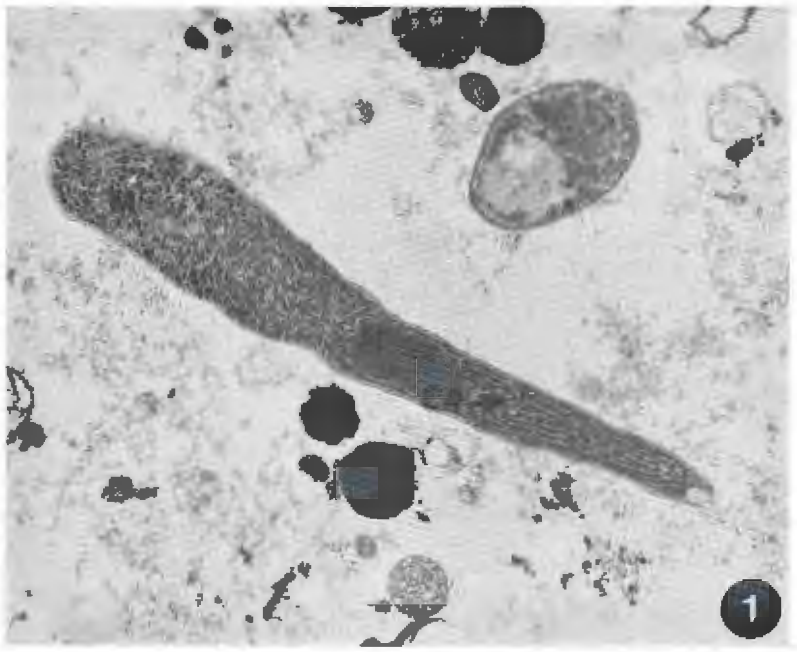

FIG. 1 Electron micrograph of a longitudinal and cross section through kinetes of *Babesia caballi* from haemolymph collected from an adult female during egg laying. Note the nucleus (N), anteriorly concentrated micronemes (M) and extensively developed endoplasmic reticulum (ER). × 6 000

were obtained from female ticks that had fed on this horse 22 days after engorgement.

Adult ticks of the subsequent generation which fed on a susceptible horse were collected daily for 8 days post-infestation. The horse underwent a mild clinical reaction with a slight increase in temperature on Day 13 post-infestation which lasted 2 days. Piroplasms were first noted in thin blood films on Day 12 after tick infestation. The highest parasitaemia was less than 1 piroplasm per 5 000 red blood cells and the animal recovered spontaneously.

Fine structure of kinetes

The kinetes observed in electron micrographs of haemolymph samples averaged 13,5 µm in length with a diameter of 2,2 µm. Kinetes were tapered, being wider at the anterior end and containing a single nucleus, abundant endoplasmic reticulum and numerous micronemes which were concentrated anteriorly (Fig. 1).

Kinetes were first observed in salivary glands of adult ticks on Day 2 post-infestation and on each subsequent day of feeding. Newly-entered kinetes were enclosed within a parasitophorous vacuole and were usually folded (Fig. 2b). They were bounded by a pellicle composed of a denser inner membrane terminating anteriorly in a polar ring and an outer plasmalemma (Fig. 2a & 3). Micropores were found along the pellicle (Fig. 2a). Thirty subpellicular microtubules were discernible in cross sections of different kinetes. A well-developed endoplasmic reticulum, micronemes, ribosomes, several mitochondria and a nucleus were also observed. Numerous kinetes were sometimes encountered within an individual acinar cell, each within a parasitophorous vacuole (Fig. 2b).

It was also noted that rickettsia-like organisms were present within the same cell in which kinetes were found (Fig. 3). They occurred individually and in large groups with each enclosed within an individual vacuole of host origin. These organisms were pleomorphic and had a multimembraned cell wall with a dense cortical region and a reticulated medullary area.

Observations on sporogony of B. caballi

Sporogony began following the disappearance of the parasitophorous vacuole with the kinete in direct contact with the host cell cytoplasm (Fig. 3). After the loss of the inner membrane of the pellicle, kinetes developed into a polymorphous sporont in which the nucleus became highly lobed and the parasite increased greatly in size (Fig 4). Following successive divisions of the nucleus and the disappearance of the apical complex and micronemes, the parasite mass appeared to be divided into numerous cytomeres with each containing at least one lobed nucleus (Fig. 4). The developing parasite was extensively folded over itself at this time and newly-entered kinetes were observed within the parasite mass (Fig. 5). Owing to the highly compact nature of the parasite mass it could not be determined if there was cytoplasmic continuity between the cytomeres. After further nuclear divisions, individual rounded nuclei were found to be encorporated into portions of cytoplasm (Fig. 6). The first sporozoites were found on Day 4 after attachment and on each subsequent day of feeding. Formation of sporozoites was not synchronous and nuclear divisions continued. Sporozoites averaged 2,5 $\frac{2}{32}$m in length and were bounded by a pellicle, with up to 4 rhoptries, micronemes and elongated mitochondria (Fig. 7). A spherical body was also observed in each sporozoite (Fig. 7).

In light microscopic observations of thick sections, kinetes and early stages of sporont growth could not be seen. The developing sporont could be seen as a compact mass filling most of the host cell on Day 3 of tick feeding (Fig. 8). The host cell nucleus was often pushed to the periphery of the cell (Fig. 8). Mature sporozoites could be easily discerned filling the host cell and, although the individual host cell was enlarged, there was no noticeable hypertrophy of the acinus (Fig. 9).

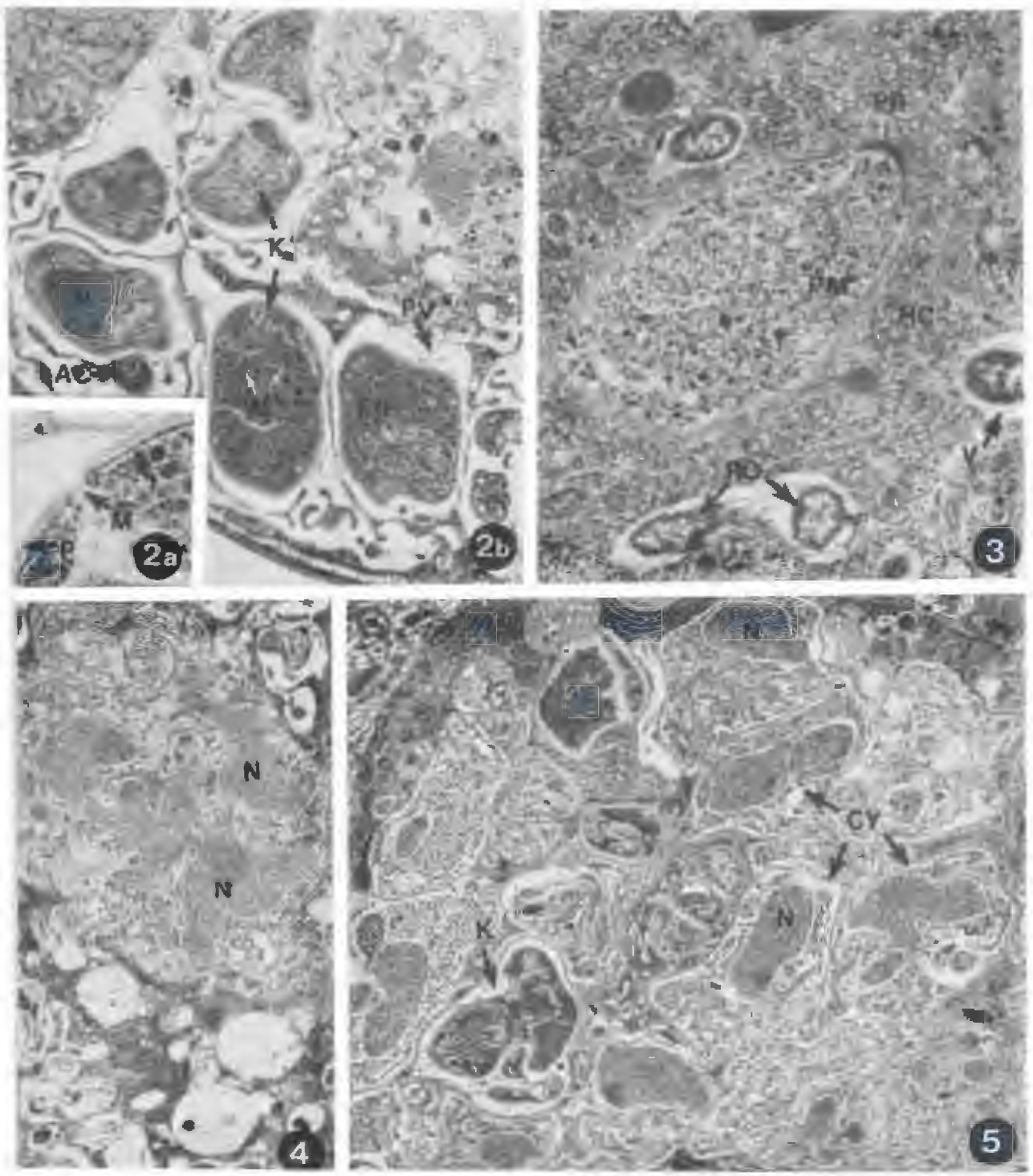

FIG. 2–5. Electron micrographs of developmental stages of *Babesia caballi* in the salivary glands of *Hyalomma truncatum*

2. Cross section through several newly-entered kinetes (K) each within a parasitophorous vacuole (PV).
   a. Pellicle of a kinete with an inner dense membrane (I), an outer plasmalemma (P) and a micropore (M). × 42 000
   b. Kinetes containing numerous micronemes (M), endoplasmic reticulum (ER), an apical complex (AC) and a nucleus (N). × 10 000
3. Longitudinal section through a kinete after the disappearance of the parasitophorous vacuole. The parasite membrane (PM) is in direct contact with the host cell cytoplasm (HC). A polar ring (PR) is still present. Note the rickettsia-like organisms (RO) in individual vaccuoles (V) within the host cell cytoplasm. × 16 800
4. Cross section through a polymorphous sporont with its highly lobed nucleus (N). × 6 720
5. Cross section through a group of cytomeres (CY) containing lobed nuclei (N). Note the newly-entered kinetes (K) within the dividing parasite mass. × 8 000

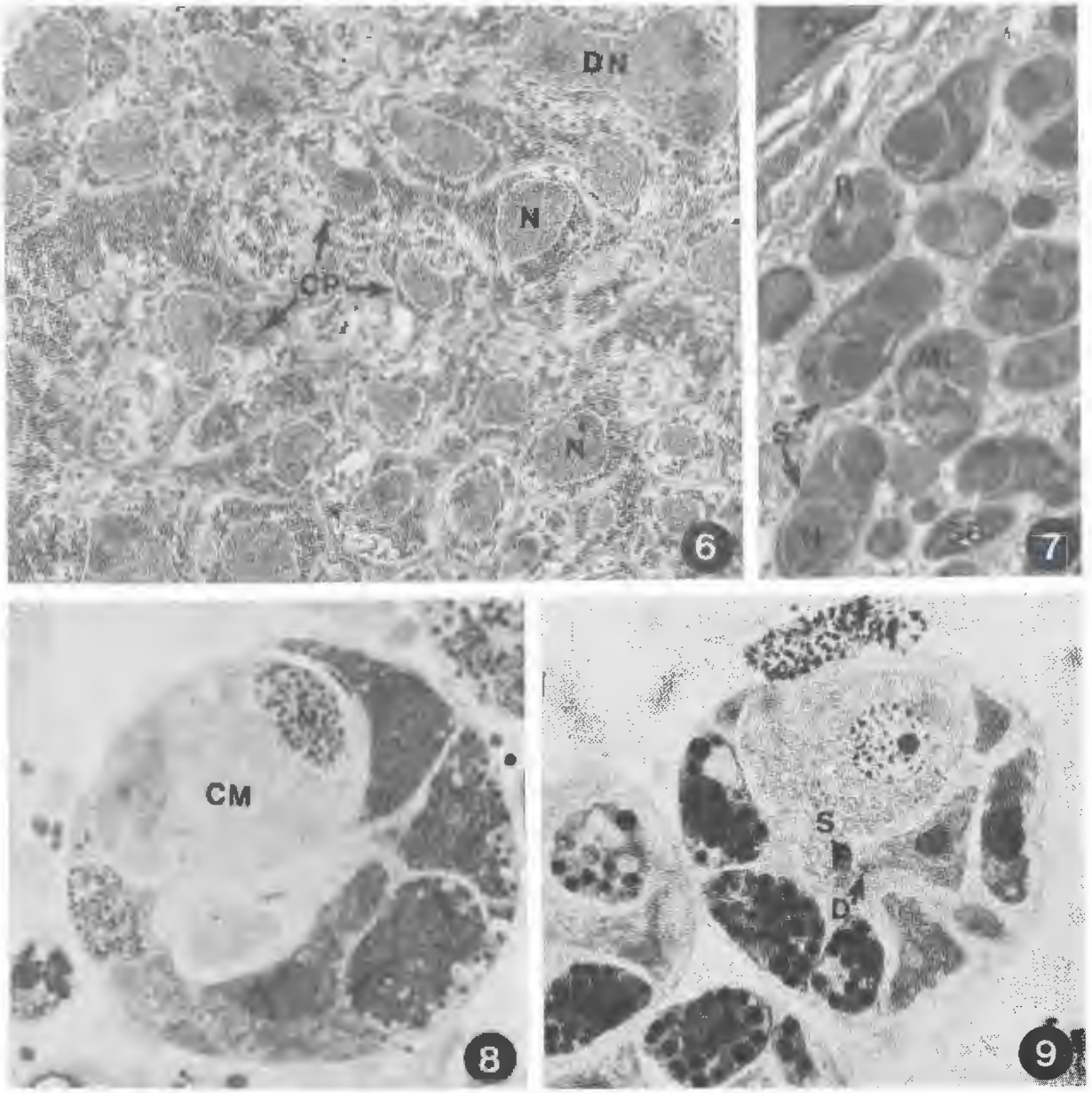

FIG. 6–7 Electron micrographs of developmental stages of *Babesia caballi* in the salivary glands of *Hyalomma truncatum*

6. Cross section through a sporont showing individual rounded nuclei (N) incorporated into membrane bound cytoplasmic portions (CP). Some larger nuclei appear to be in the process of dividing (DN). × 13 350
7. Cross section through mature sporozoites (S) containing rhoptries (R), mitochondria (Mi), spherical bodies (SB) and nuclei (N). × 8 400

FIG. 8–9 Light micrographs of developmental stages of *Babesia caballi* in the salivary glands of *Hyalomma truncatum*

8. Cross section through a sporont which appears as a compact mass (CM) enlarging the host cell. The host cell nucleus (N) is visible near the periphery of the cell. × 1 000
9. Cross section through a host cell containing mature sporozoites (S) which are entering the duct (D) of the gland. × 1 000

Parasite stages were found within granular secretory cells of the acinus (Fig. 8 & 9) and 80 % of the ticks examined between Days 2 and 8 of tick feeding contained parasitic stages.

Discussion

In previous transmission trials with *H. truncatum*, De Waal (1989) was able to transmit *B. caballi* with 40 adult ticks. The infection rate observed in *H. truncatum* in the present study was high (80 %) and tends to support the contention that this tick species is a particularly good transovarial vector of this parasite.

The developmental pattern and morphology of *B. caballi* observed here has features in common with several other species of piroplasms reviewed by Mehlhorn & Schein (1984). Synchrony of development was not seen, however, kinetes, sporonts and sporozoites were observed in ticks on the same days of feeding and sometimes within the same host cell. Moltmann, Mehlhorn & Friedhoff (1982) reported on the formation of cytomeres during the development of *B. ovis* in the salivary glands of its tick vector. Mehlhorn, Schein & Warnecke (1979) reported that *Theileria ovis* transformed to multinucleate cytomeres within the salivary glands of *Rhipicephalus evertsi evertsi*. Fawcett, Young & Leitch (1985) later showed that what had previously been considered cytomeres in several *Theileria* spp. was actually a multinucleate syncytium. This multinu-

cleate syncytium is also seen in *Babesia equi* (Moltmann, Mehlhorn, Schein, Voigt & Friedhoff, 1983) and *Babesia microti* (Karakashian, Rudzinska, Spielman, Lewengrub, Piesman & Shourkrey, 1983). The developmental stages identified as cytomeres in this study were a consistent feature and no continuity of parasite cytoplasm could be seen in the many infected acini examined. No distinct clefts containing host cell cytoplasm and organelles could be detected between the cytomeres and it is possible that the expanding sporont mass was folded over itself. The possibility of multiple infections also exists, but the large number of these groups of cytomeres observed in numerous acinar cells of many ticks and the absence of multinucleated stages make it seem unlikely that each developed from an individual kinete.

The formation of sporozoites appeared to be more of a segmentation of individual nuclei rather than as a peripheral protrusion from a multinucleated sporont. Potgieter & Els (1977) reported that during the sporogonic cycle of *Babesia bigemina* in tick salivary glands, membranes enclosed individual daughter nuclei forming immature sporozoites. The segmentation seen in *B. caballi* may simply be the result of the compact nature of the sporont mass.

The rickettsia-like organisms observed are thought to be arthropod-associated symbiotic rickettsia, as the ticks have been maintained in the laboratory on rabbits and sheep for many generations. Without serological and biochemical evaluation the exact nature of these organisms could not be determined.

This report constitutes some preliminary observations on the sporogonic cycle of *B. caballi* in *H. truncatum*. Although a basic pattern of development has been observed, further observations are needed particularly to verify the formation of true cytomeres.

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