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3

FINE STRUCTURE OF *THEILERIA PARVA* IN THE BOVINE SKIN

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ABSTRACT

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Ultrastructural studies of *Theileria parva* in the bovine skin revealed 'infective particles' of the parasite. These parasite forms were pleomorphic and were found extracellular or within host lymphoid cells, neutrophils and erythrocytes. The parasites were a product of extracellular schizogony. They were phagocytosed by the host leucocytes but seemed actively to invade the erythrocytes. Several extracellular uninucleate schizonts were also observed. The presence of extracellular infective particles, uninucleate schizonts and multinucleate schizonts, some showing schizogony, suggests an extracellular life cycle of *T. parva* within bovine tissue.

INTRODUCTION

Theileria parva is a protozoan parasite which causes East Coast Fever (theileriosis) in cattle and whose life-cycle is not yet fully understood.

Ultrastructural studies of the parasite in the bovine tissue have been performed in the lymph node (Jarret and Brocklesby, 1966; Büttner, 1967; De Martini and Moulton, 1973; Mugera and Munyua, 1973). Histopathological and ultrastructural studies of bovine cutaneous lesions have also been reported (Kimeto, 1978).

The present paper is a fine structural study of *Theileria parva* in the bovine skin lesions caused by *T. parva*-infected *Rhipicephalus appendiculatus* ticks at 120 h after tick attachment.

MATERIALS AND METHODS

Animals. Four 6-month-old Hereford calves were used. They had been purchsed 2 months before the experiment from a government farm which was free of East Coast Fever (theileriosis) and where the animals were regularly dipped using acaricides. Blood samples were collected for examination of any other haemoparasites.

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Tick attachment. A special tick holder was made from a transparent tube approximately 1.2 cm in diameter from which four small tubes of about 60 mm were cut. One of the free ends of each tube was covered with the non-adhesive side of Paragon adhesive. Two adult engorged and *T. parva*-infected ticks were placed in each holder. The calves were restrained, and tick holders were placed on the ventral side of the tail in the anal region so that the free end was on the skin. The tick holders were then secured firmly in place by tying the adhesive around the tail to cover the tick holders completely. This area was chosen because it is one of the sites for which *R. appendiculatus* has a predilection.

Skin biopsy. At 120 h after tick attachment, ticks were removed and calves were given local anaesthesia before skin biopsies were taken from anal region where ticks were attached.

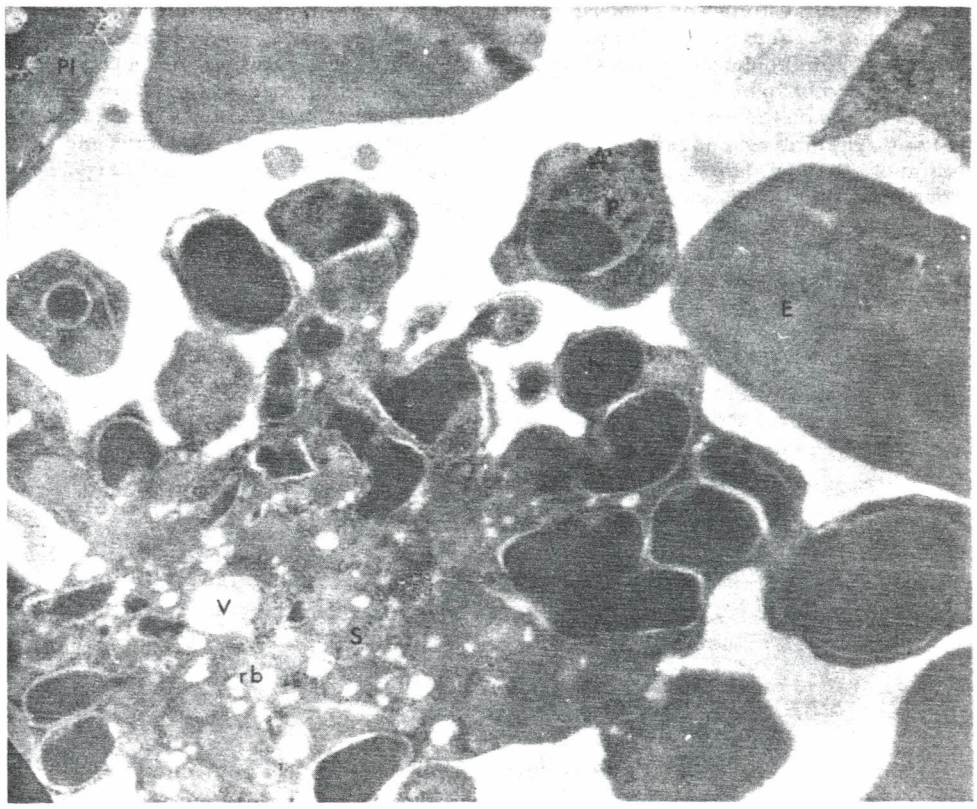


Fig. 1. Schizont (S) from an East Coast Fever-affected calf showing many nuclei (N), detached infective particles (P), residual body (rb) and vacuoles (V). Platelet (Pl. erythrocyte (E), lymphoid cell (L). (× 35,000).

Electron microscopic studies. Skin biopsy samples were fixed according to the method of Ito and Karnovsky (1968) in formaldehyde-glutaraldehyde mixture containing 0.02% trinitrocresol for 2 h at 4°C, washed three times (5 min each time) with 0.2 M phosphate buffer (pH 7.2), and post-fixed with 1% osmium tetroxide in 0.2 M phosphate buffer (pH 7.2) for 3--5 h. Blocks were washed three times (5 min each time) in isotonic saline solution, dehydrated in series of acetone, and embedded in Durcupan.

Thin sections, 50—70 nm thick, were cut, mounted on polyvinyl formol resin-coated copper grids, and stained with uranyl acetate for 8 min and with lead citrate for 5 min. The sections were then examined with an electron microscope and photographed.

RESULTS

Extracellular schizonts were observed among lymphoid cells and erythrocytes. The nuclei were electron dense and of different sizes and each was surrounded by a narrow electron lucent band and narrow cytoplasm. The number of nuclei in each schizont was four or more (Fig. 1). Other schizonts

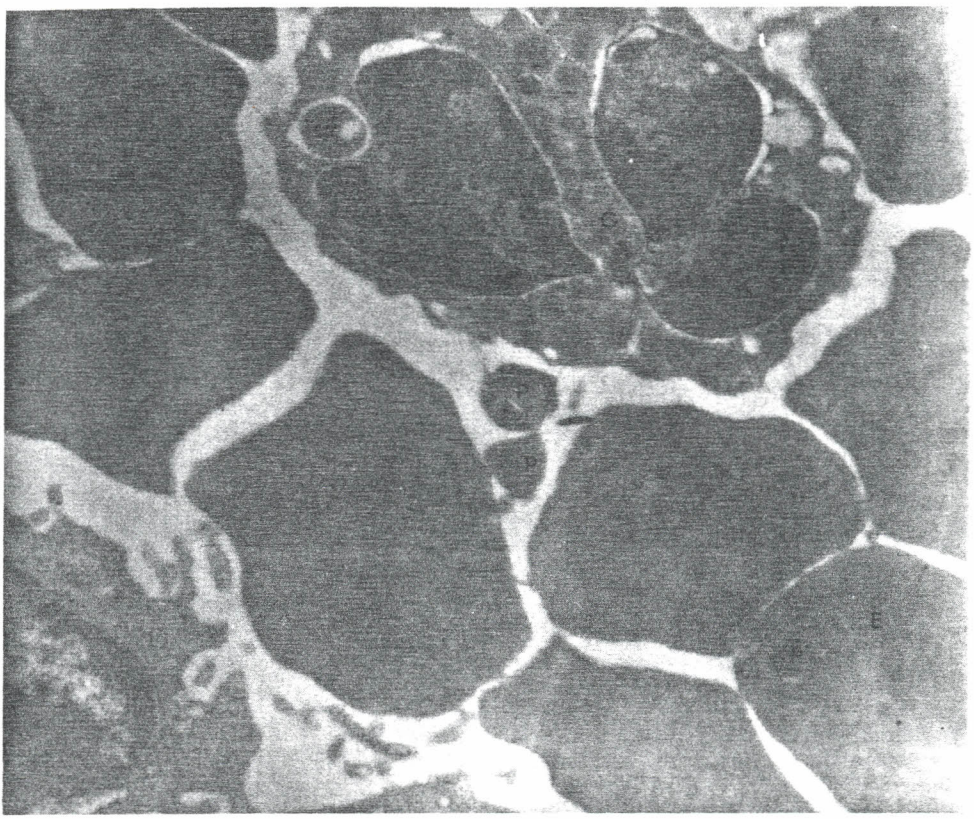


Fig. 2. Infective particle (p) within granulocyte (G) and erythrocyte (E), one making contact with erythrocyte and one cell free; Lymphoid cell (L). (× 21,000).

were observed giving off pleomorphic 'infective particles' which were mostly granulated. The term 'infective particle' was used because these parasite forms did not show clear merozoite structures like pellicle, microtubules, polar rings, micronemes and rhoptries. One such infective particle (Fig. 2) has made contact with an erythrocyte and the point of contact was electron lucent. Some infective particles were observed within the erythrocytes but the majority were either cell free or had been phagocytosed by the lymphoid cells or neutrophils.

Detached from the schizont were also large round bodies with electron dense centres (nuclei) and surrounded by narrow electron lucent bands followed by a granular surface-coat (cytoplasm). Many of them were observed and presumed to be large forms of infective particles. They were either cell free or had been phagocytosed by lymphoid cells (Fig. 3) where they came to lie within parasitophorous vacuoles. Some of the nuclei (Fig. 4) had lost their electron density and instead membranous profiles and numerous small electron dense

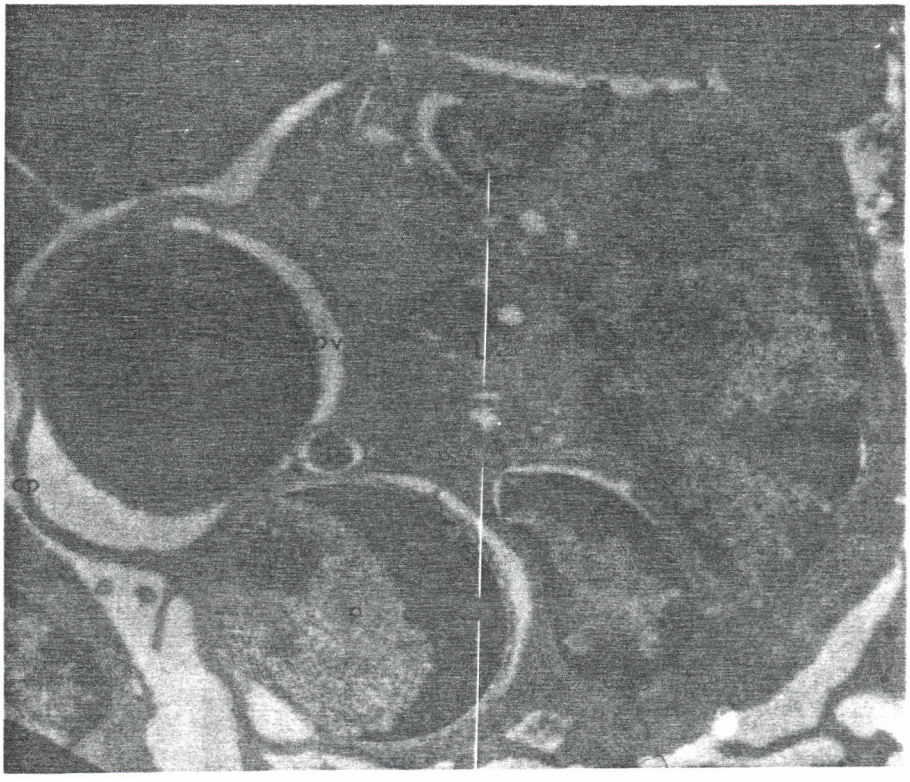


Fig. 3. Lymphoid cell (L) which has phagocytosed one small and two large 'infective particles' (p), the nuclei are at different stages of transformation. Erythrocyte (E). Cytoplasmic process (cp). Parasitophorous vacuole (pv). (× 21,000).

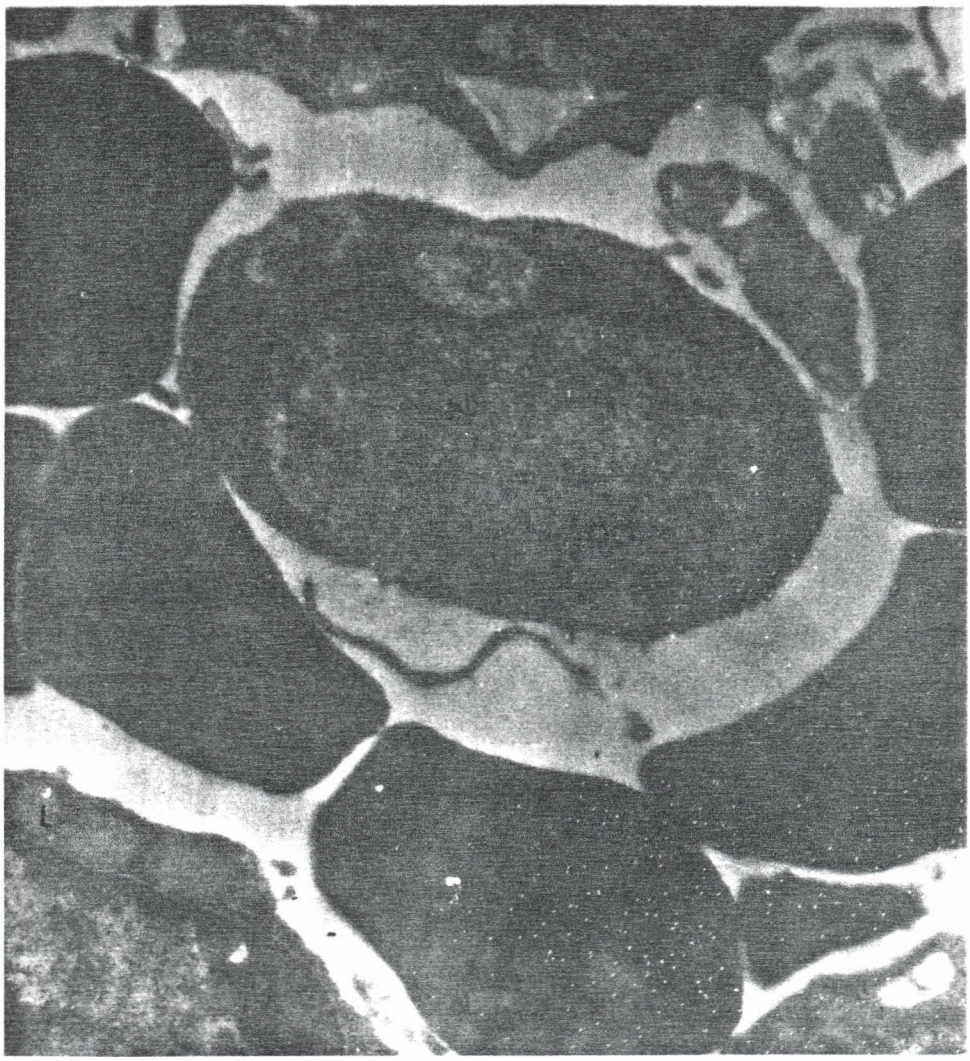
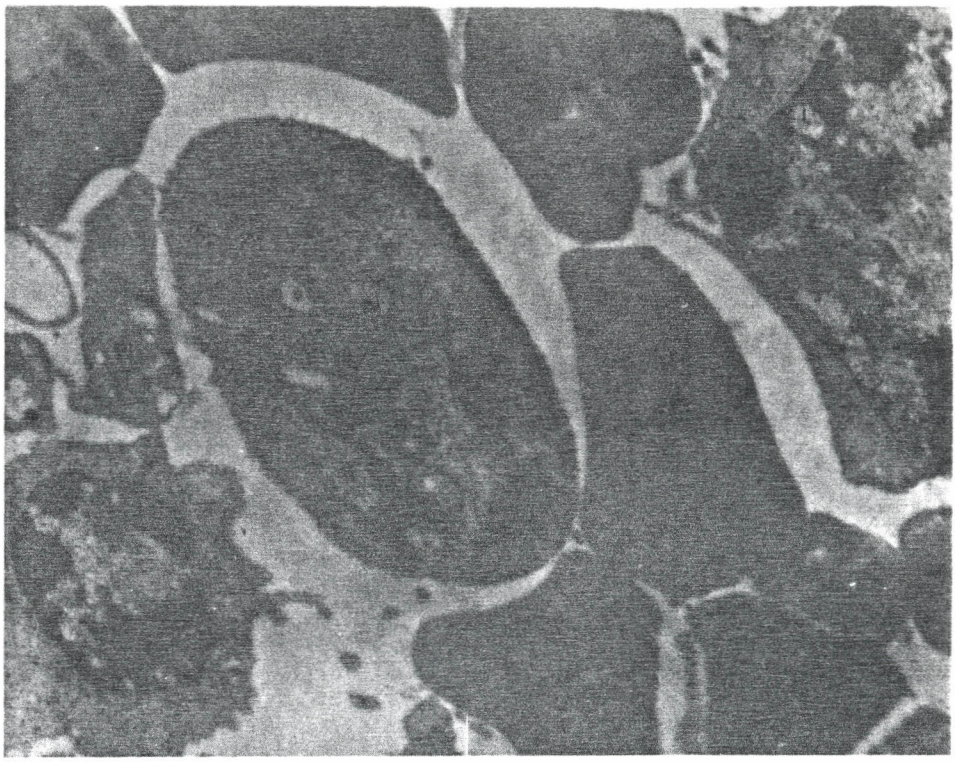
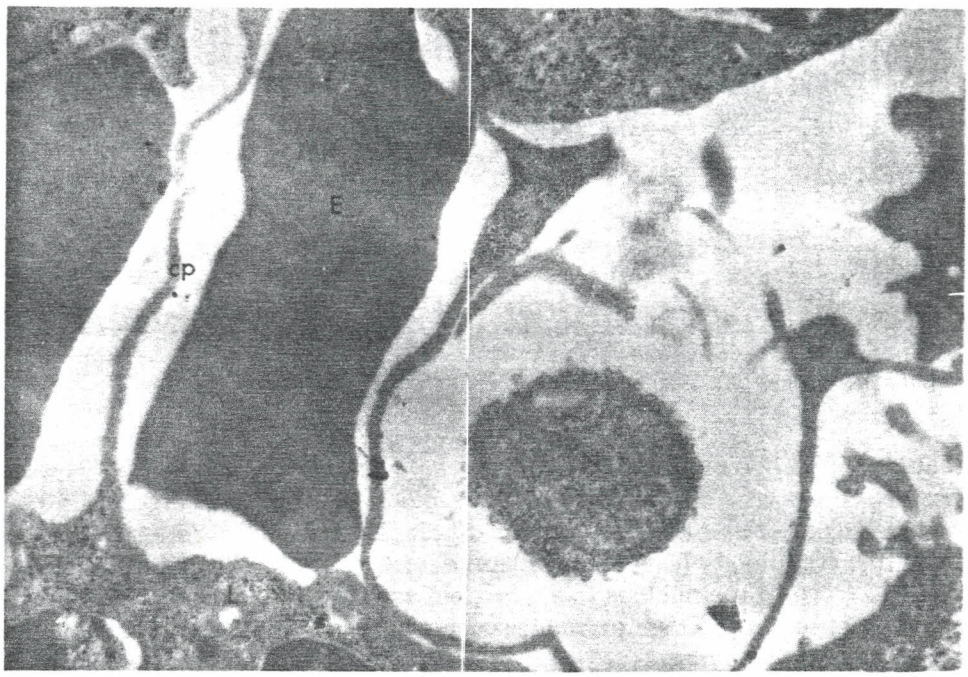


Fig. 4. Extracellular large infective particle (P). The nucleus (N) is transforming and surrounded by little cytoplasm (C). Dense intranuclear granules (DG), erythrocyte (E), lymphoid cell (L). (× 21,000).

granules were observed. A narrow and oval electron lucent band (Fig. 5) was observed in the granular surface coat (cytoplasm) demarcating an oval body, and close to the nucleus was a vacuole. Nucleolus was also observed. Some nuclei had completely lost their electron density (Fig. 6). The cytoplasm was large and close to the nucleus was a vacuole and in some parasites, a round electron dense body. These uninucleate parasites represent the early schizogonous stage of *T. parva*.




E
CP

DISCUSSION

Extracellular schizonts observed among lymphoid cells and erythrocytes had marginated nuclei, similar to those reported in the cytoplasm or lymphoid cells (De Martini and Moulton, 1973). The number of nuclei per schizont was variable but the least was four. Infective particles were phagocytosed by lymphoid cells and neutrophils. Some of these forms would presumably also be phagocytosed by the reticulum cells of the spleen where they would grow into schizonts (Moulton et al., 1971). These phagocytic cells do not destroy the parasite, perhaps because the parasite has leucocyte properties acquired by ingesting leucocyte granules and glycogen particles (Kimeto, 1978).

Infective particles approached the erythrocytes with pointed ends. Usually these forms showed little granulation. They were also smaller than the ones observed within lymphoid cells.

The parasites showed transformation of the nucleus from electron density to electron lucency. In some nuclei were electron dense granules similar to those observed by Mehlhorn et al. (1978) in the nucleus of a mature kinete of *Theileria parva*. The cytoplasm was also enlarged. These parasites, although a product of schizogony, were termed 'infective particles', because they were injected by the feeding ticks, infected the host cells, and also because they did not show clear merozoite structures, such as pellicle, microtubules, polar rings, micronemes and rhoptries.

Since infective forms, uninucleate and multinucleate schizonts and schizogony were observed extracellularly, it may be possible to postulate an extracellular life-cycle of the parasite within bovine tissue. This extracellular life-cycle is in addition to the one observed within the lymphoid cells (Büttner, 1967). The extracellular life-cycle constantly produces infective particles which infect the lymphoid cells especially in the early stages of theileriosis. During the late stages of the disease when the phagocytic activity of RHS and RES has been blocked, the parasites infect the erythrocytes in large numbers. The infection of lymphoid cells by *T. parva* is by a process of phagocytosis, and of erythrocytes by invasion.

Fig. 5. Extracellular large infective particle (P) showing transforming nucleus (N), oval body (ob) in the cytoplasm (C). Nucleolus (Nu), vacuole (v), erythrocyte (E), lymphoid cell (L), platelet (Pl). (× 21,000).

Fig. 6. Cell free uninucleate schizont showing large nucleus (N) and little cytoplasm (C). Lymphoid cell (L), erythrocyte (E), cytoplasmic process (cp). (× 21,000).

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