

FINE STRUCTURE OF *Neospora caninum* IN A WHITE RHINOCEROS CALF

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INTRODUCTION

Neosporosis is an important multisystemic disease that affects a wide variety of animals but has only recently been reported in the white rhinoceros (*Ceratotherium simum*). The disease is caused by the apicomplexan protozoal parasite, *Neospora caninum*. This paper illustrates the fine structure of the parasite in a 16 day old white rhinoceros calf that died suddenly while in excellent condition and showing no obvious previous clinical signs¹. The calf was born to a healthy mature adult female free-ranging with 11 other rhino as well as various other game species on a 2000 hectare Game Breeding Centre adjacent to the town of Lichtenburg and outlying cattle farmlands in the Northern Province of South Africa. Post mortem gross examination revealed a hypertrophic heart and signs indicative of heart failure, namely generalised cyanosis with pulmonary and hepatic congestion and oedema.

MATERIALS AND METHODS

The positive identification of the organisms in 10% formalin-fixed myocardial tissue as *Neospora* species was made light microscopically by using the immunohistochemical avidin-biotin technique², employing both polyclonal and murine monoclonal *Neospora caninum* antibodies. Tissue for electron microscopy was further prepared in 2.5% glutaraldehyde and ultrathin sections prepared for electron microscopic techniques were examined in a Philips CM10 transmission electron microscope.

CONCLUSION

The immunohistochemical and ultrastructural features of the bradyzoites containing cyst and the bradyzoites are largely consistent with those described for *Neospora caninum*, but also showed some overlap with the characteristics of *Toxoplasma gondii*, in particular the smooth nature and dimension of the cyst wall and the fact that the rhoptries were not completely electron dense, although the internal structure was indistinct.

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RESULTS

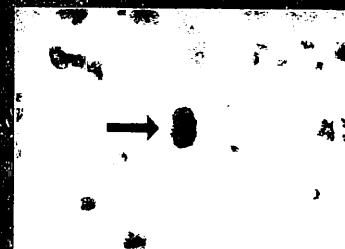


Fig. 1. High dry magnification of immunohistochemically stained bradyzoite (arrow) in the myocardium.

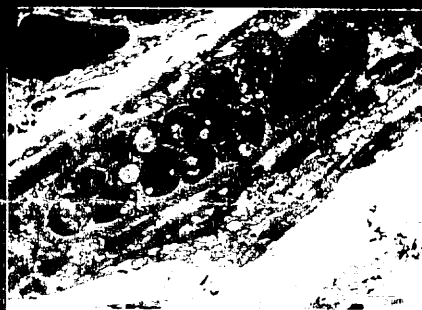


Fig. 2. Low magnification electron micrograph of intramyocardial encysted bradyzoites. Cyst wall (C), bradyzoites (B), myofibrils (M).



Fig. 3. An enlarged region of the intracellular cyst illustrating the 0.33µm wide cyst wall (C), consisting of an even-layered paracrystalline vascular membrane and a thick underlying granular layer. Cross-sections of rhoptries (R) equidistantly situated (M), dense granules (G), vesiculae and amylopectin granules (A) are also visible. Note the perpendicular arrangement of the micronemes (M) to the zeta pole (Z).

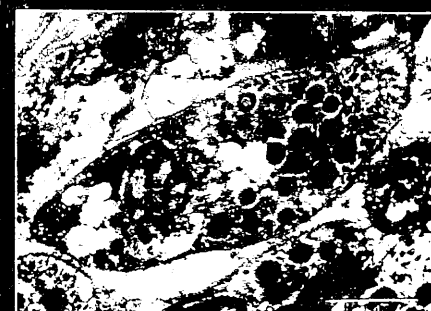


Fig. 5. Higher magnification demonstrating a longitudinally sectioned zeta measuring 4.5 µm and containing a subterminal nucleus (N), lipid bodies (L), dense granules (G), rhoptries (R) and micronemes (M).

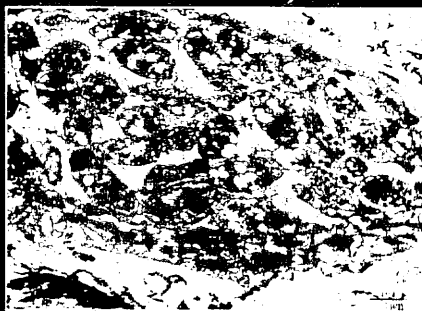


Fig. 4. Low magnification micrograph of bradyzoite (B) lying free within the host cell cytoplasm. Note a dividing zeta (*)



Fig. 6. An enlargement of a bradyzoite showing the canal (C) and a vesiculo-membranous organelle (V), rhoptries (R), dense granules (G) and micronemes (M).



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MATERIALS AND METHODS

The positive identification of the organisms in 10% formalin-fixed myocardial tissue as *Neospora* species was made light microscopic by using the immunohistochemical staining technique employing both polyclonal murine monoclonal *Neospora caninum* antibodies. Tissue for transmission electron microscopy was further prepared by glutaraldehyde and ultrathin sections prepared by the conventional microscopic technique. The electron microscope used was a Philips CM10 transmission electron microscope.

CONCLUSION

The immunohistochemical and ultrastructural features of the tachyzoite-containing cyst and the tachyzoites are largely consistent with those reported for *Neospora caninum*, but also showed some overlap with the characteristics of *Toxoplasma gondii*, in particular the smooth nature and dimension of the cyst wall and the fact that the rhoptries were not completely electron dense, although the internal structure was indistinct.

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RESULTS

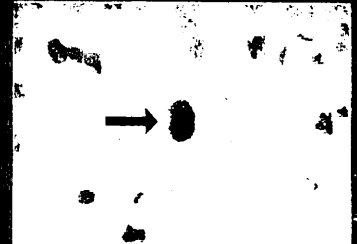


Fig 1. Higher magnification of immunohistochemically stained tachyzoites (arrow) in the myocardium.



Fig 2. Low magnification electron micrograph of intramyocardial encysted tachyzoites. Cyst wall (C), tachyzoites (B), myofibrils (M).

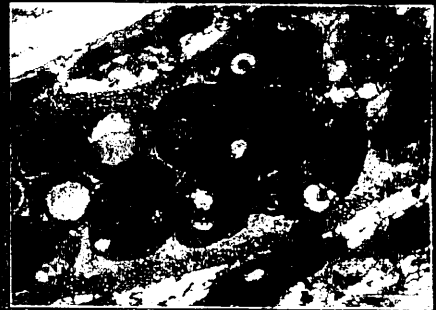


Fig 3. An enlarged region of the intracellular cyst illustrating the 0.35µm wide cyst wall (C) consisting of an evenly-thick parasitophorous vacuolar membrane and a thick underlying granular layer. Cross-sections of tachyzoites (B) revealing a nucleus (N), dense granules (G), amylopectin granules (A) are also visible. Note the perpendicular arrangement of the micronemes (M) to the zeta pole (Z).

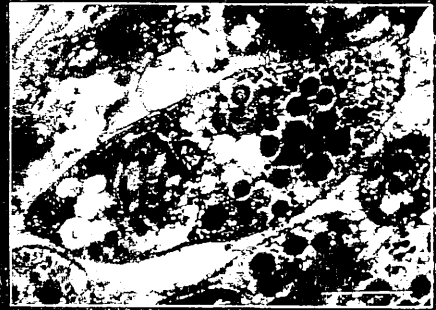


Fig 5. Higher magnification demonstrating a longitudinally sectioned zone containing 4.8 x 2µm and containing a subterminal nucleus (N), lipid bodies (L), dense granules (G), rhoptries (R) and micronemes (M).



Fig 4. Low magnification micrograph of tachyzoites (T) lying free within the host cell cytoplasm. Note a dividing zeta (Z).

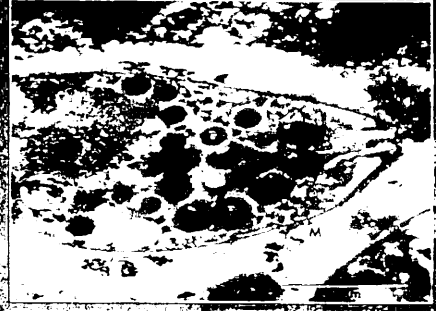


Fig 6. An enlargement of a tachyzoite showing the conoid (C) and a vesiculo-membranous organelle (V), rhoptries (R), dense granules (G) and micronemes (M).

