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RESEARCH NOTE

THE TRANSMISSION OF *BABESIA BOVIS* USING FROZEN INFECTIVE MATERIAL OBTAINED FROM *BOOPHILUS MICROPLUS* LARVAE

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ABSTRACT

POTGIETER, F. T. & VAN VUUREN, A. S., 1974. The transmission of *Babesia bovis* using frozen infective material obtained from *Boophilus microplus* larvae. *Onderstepoort J. vet. Res.*, 41, ... (1974)

Boophilus microplus larvae infected with *Babesia bovis* were removed from a susceptible ox approximately 72 hours after attachment, triturated and stored in a liquid nitrogen refrigerator with dimethyl sulphoxide as cryoprotectant. When inoculated into splenectomized cattle the suspension was infective in the 2 animals into which it was injected intravenously but not in the 3rd, which was only injected subcutaneously. The prepatent period in the infected cattle was 10 and 11 days respectively.

Cryopreservation of erythrocytic stages of several *Babesia* spp. has become standard practice in some laboratories (Waddell, 1963; Barnett, 1964; Pipano & Senft, 1966; Frerichs, Johnson & Holbrook, 1968; Dalgliesh, 1971, 1972b; Bishop, Adams, Thomson & Corrier, 1973). To our knowledge, however, no reports exist on the successful preservation of the tick stages of any *Babesia* spp.

During studies on the life cycle of *Babesia bovis* (Potgieter & Van Vuuren, unpublished observations, 1973), the highest numbers of the terminal developmental stage of the organism in *Boophilus microplus* larvae (viz. the infective particles, described by Riek, 1965) were found to be present in the ticks 72 hours after infestation of the bovine host. Thereafter a marked decline in the numbers of these infective particles was noted. These findings agree with those of Riek (1965) and Mahoney & Mirre (1971) regarding the development of *Babesia argentina* [which is thought by Riek (1968) to be a synonym of *B. bovis*] in *B. microplus* larvae. Following these observations an attempt was made to store the infective stage of *B. bovis* from ticks, using information obtained from studies on the cryopreservation of tick tissues infected with *Theileria parva* by Wilde, Brown, Hulliger, Gall & Macleod (1968) and Cunningham, Brown, Purnell & Branagan (1970).

Larval ticks from a laboratory-maintained strain of *B. microplus* infected with *B. bovis* were fed on a fully susceptible, splenectomised ox (no. 9225) which had been raised and maintained under tick-free conditions. Seventy-two hours after infestation 20 partially engorged larvae were removed, their mouth parts and legs severed and their bodies triturated in a tissue grinder for 10 minutes at 4°C in 5 ml of diluent. This diluent consisted of equal volumes of dimethyl sulphoxide (DMSO) and a phosphate buffered saline solution containing 2% disodium ethylenediamine tetra-acetate (EDTA), diluted to 1 in 4 with sterile bovine serum obtained from a susceptible splenectomised donor. Four 1 ml ampoules were filled with the chilled suspension, sealed hermetically and transferred immediately to the gas phase of a liquid nitrogen refrigerator.

The infectivity of the frozen material was determined after 8–12 weeks storage by inoculating it into 3 susceptible, splenectomised oxen, raised under tick-free conditions. The material was removed from the refrigerator, thawed rapidly at approximately 30°C to obviate possible toxic effects of DMSO (Dalgliesh, 1972a) and injected immediately. Thick and thin bloodsmears were prepared daily and stained with Giemsa. The animals' rectal temperatures were recorded daily.

Ox 9387 was injected with 2 ml of the suspension, 1 ml intravenously and 1 ml subcutaneously. Typical *B. bovis* parasites were first seen in thick bloodsmears taken on Day 11 after inoculation. During the ensuing 5 days the percentage parasitaemia increased rapidly and was accompanied by a febrile reaction. On Day 16 after inoculation chemotherapy was deemed necessary and the reaction terminated following an injection of diminazene*.

To confirm the diagnosis of *B. bovis* infection a brain biopsy was performed immediately prior to chemotherapy and brain smears prepared as described by Johnston & Callow (1963). Eighty-four per cent of the erythrocytes in the brain capillaries were found to be infected with *B. bovis*, compared to a parasitaemia of 1% observed in peripheral blood. This predilection for brain capillaries is a distinctive feature of *B. argentina* infections (Hoyte, 1971; Wright, 1971; Callow & Johnston, 1963) and has also been observed in *B. bovis* infections at this institute (A. de Vos, unpublished observations, 1973).

Ox 9486 became infected after the intravenous inoculation of 1 ml of the frozen material. The prepatent period in this case was 10 days and again a typical primary reaction followed. On Day 14 the animal had a morning temperature of 40,5°C and a peripheral blood parasitaemia of 0,2%. The reaction in this case terminated following treatment with imidocarb**.

Ox 9499 was inoculated with 1 ml of the suspension subcutaneously. No evidence of infection could be detected in this animal over an observation period of 60 days. To exclude the possibility that it had developed a subpatent infection, an attempt was made to induce a patent relapse by injecting 0,05 mg/kg prednisolone*** intramuscularly for 5 days starting on Day 64 (Callow & Parker, 1969). A brain biopsy was performed on the animal on Day 73, but no parasitized red cells were found during a 30 minute examination of the smears.

Although these results suggest that the inoculum was only infective when injected intravenously, further research will be necessary to confirm and establish the reason for the lack of infectivity following subcutaneous injection.

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* Berenil, Hoechst.
** Imizol, Burroughs Wellcome & Co.
*** Delta-Cortril, Pfizer Laboratories Ltd., R.S.A.

Since the infective particles were the only developmental stage of *B. bovis* seen in the larvae 72 hours after infestation of the host, it is reasonable to assume that they had survived freezing and were responsible for infection.

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