

RESEARCH COMMUNICATION

ISOLATION OF *ANAPLASMA MARGINALE* FROM *RHIPICEPHALUS SIMUS* MALES

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ABSTRACT

POTGIETER, F. T. & VAN RENSBURG, L., 1980. Isolation of *Anaplasma marginale* from *Rhipicephalus simus* males. *Onderstepoort Journal of Veterinary Research*, 47, 285-286 (1980).

Approximately 100 adult *Rhipicephalus simus* from a batch known to be infected with *Anaplasma marginale* were used to infest an ox. Fifty male ticks were manually removed from the animal's ears 9 days after infestation. These ticks were triturated and a stabilate was prepared which was injected intravenously into 2 susceptible oxen. Both these animals became infected with *A. marginale*. The prepatent periods following inoculation of the tick suspensions before and after freezing in liquid nitrogen were 16 and 17 days respectively.

Résumé

ISOLATION D'ANAPLASMA MARGINALE DE MALES DE RHIPICEPHALUS SIMUS

Approximativement 100 *Rhipicephalus simus* adultes d'un lot reconnu comme étant infecté avec *Anaplasma marginale* ont été utilisés pour infester un boeuf. Cinquante tiques mâles furent retirées manuellement des oreilles de l'animal neuf jours après l'infestation. Ces tiques furent broyées et un stabilat fut préparé et fut injecté par voie intra-veineuse à deux boeufs susceptibles. Les deux animaux devinrent infectés avec *A. marginale*. Les périodes prépatentes suivant l'inoculation des suspensions de tiques avant et après congélation dans l'azote liquide furent de 16 et 17 jours respectivement.

INTRODUCTION

Apart from the observations made by Theiler (1910, 1911) on the transmission of anaplasmosis in South Africa, no other reports have been made on the experimental biological transmission of the disease in this country.

A project was recently undertaken to investigate this particular aspect of the epizootiology of the disease. One important observation made during this study was that the 3-host tick *Rhipicephalus simus* can act as an efficient transstadial vector of *Anaplasma marginale*. Transmission in this tick species takes place from larvae to nymphae and from both larvae and nymphae to adults. The latter implies larval exposure to infection and subsequent transmission of this infection to the bovine host by both the nymphae and the adults of the same batch of ticks, without the engorging nymphae being exposed to a patent parasitaemia in the host (Potgieter, unpublished observations, 1979).

TRANSMISSION OF *A. marginale* IN THE LABORATORY

Tick transmission

A hundred adult ticks from a batch of a laboratory-maintained strain of *R. simus* were fed on a susceptible splenectomized ox. Part of this particular batch of adult ticks had already been used to demonstrate transstadial transmission of this parasite from the nymphae to the subsequent adults. The nymphae had engorged on a splenectomized ox undergoing a primary *A. marginale* reaction.

Engorged female ticks started to drop on Day 6 and by Day 8 a total of 26 had been collected. On Day 9 approximately 50 male ticks were removed by hand and used for the preparation of a stabilate.

The ox contracted an *A. marginale* infection which showed a prepatent period of 20 days after initial tick infestation.

Tick stabilate

The 50 male ticks removed from the host were triturated in a tissue homogenizer in 8 ml of the medium used for culturing baby hamster kidney cells, as described by Macpherson & Stoker (1962). This culture medium was supplemented with 3,4% bovine plasma albumin (BPA) fraction V, as described by Purnell, Brown, Cunningham, Burridge, Kirimi & Ledger (1973).

A 20% dimethyl sulphoxide (DMSO) solution in foetal calf serum was prepared as a cryoprotectant and mixed with the tick suspension in a 1:1 ratio to bring the final concentration of DMSO to 10% before being stored in the liquid nitrogen.

To test the infectivity of the fresh material, 1,5 ml (approximately 6 ticks per 1 ml) without DMSO was injected intravenously to infect a susceptible splenectomized ox.

A total volume of 12 ml (approximately 3 ticks per 1 ml) was finally made up into 6 × 2 ml aliquots and put into the gas phase of a liquid nitrogen refrigerator.

After the stabilate had been stored for 3 weeks at low temperature, 2 ml was rapidly thawed at ±37 °C and immediately injected intravenously into a susceptible splenectomized ox.

Both animals contracted *A. marginale* infections. The prepatent periods were 16 and 17 days respectively.

DISCUSSION

This is the first observation made in South Africa of transstadial transmission of *A. marginale* by a multi-host tick. Details of the transmission studies, still in progress, will be published later. This biological transmission of *A. marginale* is confirmed by the

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successful preparation of a tick stabilate, as described above. The fact that the stabilate was prepared from male ticks indicates that migrating male ticks may play an extremely important role in the intrastadial transmission of the infection.

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