

## BABESIA, THEILERIA AND ANAPLASMA SPP. INFECTING SABLE ANTELOPE, HIPPOTRAGUS NIGER (HARRIS, 1838), IN SOUTHERN AFRICA

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### ABSTRACT

THOMAS, SHAN E., WILSON, D. E. & MASON, T. E., 1982. *Babesia*, *Theileria* and *Anaplasma* spp. infecting sable antelope, *Hippotragus niger* (Harris, 1838), in southern Africa. *Onderstepoort Journal of Veterinary Research*, 49, 163-166 (1982).

Some observations are recorded on blood parasites of sable antelopes. Blood smears of 124 of these antelopes from South Africa and Zimbabwe were examined and 7 were found to be positive for a *Babesia* sp., identified as *Babesia irvinesmithi* Martinaglia 1936. A total of 70 of the smears were positive for theilerial piroplasms, while 1 smear had macroschizonts (with cytomeres) and microschantzons of a *Theileria* (= *Cytauxzoon*) sp. One blood smear was positive for an *Anaplasma* sp.

Attempts to isolate the *Babesia* sp. by subinoculating blood from sable to splenectomized and intact sable and splenectomized cattle were unsuccessful. Attempts to infect sable with *Babesia bovis* and *Babesia bigemina* were likewise unsuccessful. Theilerial piroplasms reached high levels in a splenectomized sable but could not be transmitted with blood to cattle. The *Anaplasma* sp. was found to be infective for sheep but not for cattle.

### INTRODUCTION

African antelope species are known to be hosts of a variety of intra-erythrocytic parasites (Neitz, 1957; Löhr, Ross & Meyer, 1974; Karstad, 1979). The sable antelope, *Hippotragus niger* (Harris, 1838), is regarded as one of the rare species and it is perhaps for this reason that an extensive study of this animal had not been undertaken to date.

However, even in the case of this species, there are references to the finding of blood parasites dating back as far as 1930. Martinaglia (1930) reported the death of a sable antelope in the Johannesburg Zoological Gardens, which he ascribed to a heavy infection with a *Babesia* sp., later named *Babesia irvinesmithi* (Martinaglia, 1936).

*Theileria*-like piroplasms have also been reported in erythrocytes of Transvaal sable antelope by Neitz (1957). Carmichael & Hobday (1975) found that 4 out of 11 sable examined in Botswana harboured *Theileria*-like piroplasms. Reference to the possible pathogenicity of a *Theileria* (= *Cytauxzoon*) sp. in neonatal sable was made by Wilson, Bartsch, Bigalke & Thomas (1974).

Carmichael & Hobday (1975) found intra-erythrocytic bodies morphologically very similar to *Anaplasma* bodies in 2 out of 11 sable from northern Botswana, but they considered these to be non-parasitic in origin.

A research programme initiated by the Transvaal Nature Conservation Division to identify adverse factors affecting roan and sable antelope populations in nature reserves throughout the Transvaal (Wilson *et al.*, 1974) provided much of the field material on which these observations are based. This paper serves further to describe experiments performed on 2 captive sable antelope calves (SA 25 & 26).

### MATERIALS AND METHODS

#### Experimental animals

Blood smears of a total of 124 sable antelope in various game reserves in the Transvaal and Zimbabwe were examined for protozoan parasites. All age groups were sampled, from new-born to fully mature animals. Two 3-month-old male sable calves (SA 25 and 26) were brought from the Percy Fyfe Game Reserve in the Northern Transvaal to this Institute and kept in a small, tick-free enclosure. After a 4-week period of adaptation, during which they were examined for any natural infections, one of the calves (SA 26) was splenectomized.

Several sable were also kept in enclosures in the various game reserves to facilitate attempts to demonstrate intra-uterine transmission of *Theileria* (= *Cytauxzoon*) piroplasms (Wilson & Thomas, unpublished observations, 1976). A total of 8 splenectomized bovines and 1 sheep, which had been reared and maintained at Onderstepoort under tick-free conditions, were used.

#### Ticks and tick haemolymph smears

Large numbers of *Boophilus decoloratus* were detected on the sable calves upon their arrival at this Institute. Fifty fully engorged females were collected and kept in an acaridarium at 26 °C and 80% RH. Haemolymph smears of these ticks were prepared as described by Burgdorfer (1970), fixed in methanol and then stained with 10% Giemsa's stain for 30 min. Conventional light microscopy was used for examining the smears for protozoan parasites.

#### Transmission experiments

*Experiment 1:* In an attempt to transmit a *Babesia* sp. with *B. decoloratus* from sable to a splenectomized bovine, the larval progeny of 10 engorged female ticks collected from the sable calves (SA 25 & 26) on arrival at Onderstepoort were fed on a splenectomized ox.

*Experiment 2:* To infect SA 25 and 26 with a sable *Babesia*, 20 ml blood samples were collected in heparin from 5 sable antelopes at Percy Fyfe Game Reserve, pooled, and injected in equal volumes intravenously.

*Experiment 3:* To attempt transmission of a sable *Babesia* to cattle by blood inoculation, 100 ml of blood, collected from sables SA 25 and 26 in ACD, was injected intravenously into 2 splenectomized oxen respectively.

*Experiment 4:* In an attempt to infect the sable calves SA 25 and 26 with *Babesia bigemina*, heavily infected blood was collected in ACD from an ox and 1 ml injected subcutaneously into each of the calves. One month later 200 ml of blood was collected in ACD from each of the calves and inoculated intravenously into 2 splenectomized oxen respectively.

*Experiment 5:* The procedure described in Experiment 4 was repeated subsequently with bovine blood infected with *Babesia bovis*.

*Experiment 6:* The sable calf SA 25 had a relapse of *Anaplasma* after splenectomy and, at the peak of the reaction, blood was collected in ACD and 25 ml inoculated into each of a splenectomized bovine and a splenectomized sheep.

#### Evaluation of infections

Thin blood smears were examined from 124 sable antelope. These were prepared from peripheral blood of

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animals either immobilized for examination or else from animals found dead in the reserves. Thin blood smears, prepared from peripheral blood, were made daily of the experimental oxen, sheep and sable calves. The smears were air-dried, fixed in methanol and then stained with 10% Giemsa's stain for 30 min. Parasitaemias of intra-erythrocytic *Babesia*, *Theileria* and *Anaplasma* infections were expressed as a percentage. Theilerial schizont numbers were not quantified.

Rectal temperatures of experimental animals were recorded daily.

#### Measurement of piroplasms and schizonts

Parasites were measured by taking photographs at a fixed magnification of piroplasms, schizonts and an object micrometer\*. These photographs were enlarged 5 times and then comparative measurements were made, using the photograph of the micrometer as a scale as described by Thomas & Mason (1981).

#### Serology

Serum samples of the 2 experimental sable as well as of a bovine and a sheep inoculated with *Anaplasma*-infected blood were tested for antibodies against *Anaplasma marginale* in the complement fixation test.

## RESULTS

#### *Babesia* sp.

**Field observations:** Of the 124 blood smears of sable examined, 7 were found to be positive for a *Babesia* sp. Five of the smears were from animals which had been found dead in the field. The level of *Babesia* parasitaemia varied from < 0,01–4,4%.

A brain smear made from one of the dead animals showed large numbers of parasitized erythrocytes packing the capillaries.

**Laboratory investigations:** No evidence of a natural *Babesia* infection was found in the sable calves SA 25 and 26. Even after SA 26 was splenectomized, there was no indication of an existing infection. In addition, pooled blood samples from 5 sable at the Percy Fyfe Game Reserve, when injected into the 2 sable calves, failed to produce microscopic *Babesia* parasitaemias.

The haemolymph smears of 50 engorged *Boophilus decoloratus* females collected from the sable calves did not show the presence of any *Babesia*-type merozoites (vermicules), neither did the ensuing generation of larvae of 10 females transmit any parasites to a bovine.

One month after the injection of *B. bovis*-infected blood, one erythrocyte containing a pair of parasites resembling *B. bovis* was observed in the splenectomized sable (SA 26). The other calf (SA 25) remained negative throughout. The inoculation of 200 ml of blood from each of the sable into 2 oxen respectively 1 month after injection, however, failed to transmit *B. bovis*. It was therefore concluded that the parasites seen in the blood smear of SA 26 were not *B. bovis*.

After the injection of *B. bigemina*-infected blood into the 2 sables, blood smears remained negative and cattle injected 1 month later with blood from these animals failed to develop a *B. bigemina* infection.

**Morphology:** Parasites observed in the naturally infected cases were randomly situated within the erythrocytes. Single organisms were most commonly encountered, but occasional dividing forms and pairs were also seen. The

parasites varied from round to oval to somewhat irregular or pear-shaped, usually with a single area of purple staining chromatin. This chromatin material appeared as a single round area or a "halo" situated along a portion of the margin of the organism. The cytoplasm was blue in colour.

The parasites observed in the live sable were 1,5–2 × 0,7–1,5 μm (mean 1,58 × 1,11 μm) in size. *Theileria* (=Cytiauxoon) sp.

**Field observations:** Of the 124 field smears, 70 were positive for small theilerial piroplasms in the erythrocytes. The level of parasitaemia varied from < 0,01–14,4%.

Schizonts were found in 1 animal only, a calf with a piroplasm parasitaemia of 14,4%.

All age groups were found to be infected, although mortalities associated with high parasitaemias were found only amongst the calves. Older animals appeared to serve only as carriers, with low level parasitaemias. A new-born calf was also found to be infected.

**Laboratory investigations:** On their arrival at the laboratory, both SA 25 and 26 had low level *Theileria* parasitaemias. After splenectomy the parasitaemia in SA 26 increased to 16%, at which level this animal was treated with primaquine phosphate BP\* given twice at a daily dosage of 0,5 mg/kg. The parasitaemia subsequently decreased to 1% within a week.

**Morphology:** Piroplasms appeared in blood smears as intra-erythrocytic, polymorphic, round, oval or comma-shaped bodies. The cytoplasm stained blue and the nucleus, which was usually situated at one end or as a "halo" on the margin, stained a deep purplish-blue. Reproducing forms appeared as so-called "Maltese crosses" (with 4 daughter organisms). Although erythrocytes usually contained only 1 or 2 parasites, up to 5 per cell were observed. The length of individual parasites ranged from 1,5–1,8 μm.

A small number of macroschizonts were seen in the cytoplasm of leucocytes in a blood smear of 1 animal. They appeared as bluish bodies measuring 7–15 × 6–13 (mean 9,6 × 7,4) μm with 8–49 (mean 15) purple nuclei. Cytomeres were seen in some of the macroschizonts. A small number of microschizonts were also seen in this blood smear. These consisted of a blue cytoplasm with small nuclei arranged in groups of 10–33 (mean 19). The macroschizont index was not determined.

#### *Anaplasma* sp.

**Field observations:** Only 1 out of the 124 field smears was positive for *Anaplasma* sp. This was a smear of a sable from the Loskop Dam Nature Reserve. The parasitaemia was < 1%.

**Laboratory investigations:** After splenectomy, SA 26 developed a parasitaemia which reached a level of 12%. Chemotherapy at this stage, using oxytetracycline\*\* as a single dose of 10 mg/kg i.v., brought the parasitaemia down to 2%.

Blood, taken at the height of the reaction and injected into a splenectomized bovine and a splenectomized sheep, failed to produce clinical reactions. Marginal bodies were seen in very low numbers in erythrocytes of the sheep from the 4th week after inoculation. None were seen in the bovine.

\* Primaquine (ICI)

\*\* Terramycin (Pfizer)

\* Carl Zeiss, Jena

The complement fixation test, using *A. marginale* as antigen, demonstrated the presence of antibodies in the sheep and splenectomized sable calf. The non-splenectomized sable calf and the splenectomized bovine gave negative results in the test.

**Morphology:** This parasite was morphologically similar to *Anaplasma marginale* of cattle and approximately 80% of the bodies were situated on the margin of the erythrocytes.

## DISCUSSION

### *Babesia*

The *Babesia* sp. observed in this study was morphologically identical with that described by Martinaglia (1930) and later called *B. irvinesmithi* by Martinaglia (1936). The parasites were smaller than *B. bigemina* (Zwart, Van den Ende, Kouwenhoven & Buys, 1968; Potgieter, 1977) and *Babesia major* (Zwart *et al.* 1968; Brocklesby & Barnett, 1970), but similar in size to *B. bovis* (Neitz, 1941; Riek, 1966; Potgieter, 1977).

Neitz (1965) suggested that the relationship between this parasite and *B. bovis* be investigated. We found certain similarities; not only were the 2 species similar in size but both this *Babesia* sp. and *B. bovis* (Hoyte, 1971) had a tendency to accumulate in the brain capillaries, thereby distending the capillaries with parasitized cells.

Attempts to infect sable with *B. bovis* and *B. bigemina* were unsuccessful, as the failure to transmit these species back to cattle 1 month after attempted infection proved. We therefore assume that the sable antelope is incapable of carrying the bovine *Babesia* spp. for any length of time, and it is considered unlikely that this antelope could play any role in the epidemiology of bovine babesiosis.

We concluded from these results that the *Babesia* sp. observed was *B. irvinesmithi* and that this must therefore be considered to be a valid species of the sable antelope.

### *Theileria* (=Cytiauxzoon)

The taxonomic position of the genus *Cytiauxzoon* has been the subject of much speculation in recent years. The genus was created by Neitz & Thomas (1948) and differed from the related genus *Theileria* in that, amongst others, schizogony took place in histiocytes rather than in lymphocytes. The intra-erythrocytic piroplasms of the 2 genera were indistinguishable (Neitz, 1957). Levine (1971) found it unacceptable that the nature of the host-cell of the schizont be the criterion used to differentiate the genera and preferred to consider *Cytiauxzoon* as a synonym of *Theileria*. Brocklesby (1978), however, argued strongly for the retention of *Cytiauxzoon* and emphasized that the identity of the host-cell was not the only difference between the 2 genera.

Grootenhuis (1979) and Grootenhuis, Young, Dolan & Stagg (1979) studied a theilerial parasite of the eland, *Taurotragus oryx*. A stabulate of this parasite, prepared from ticks that fed on an infected eland, was found to be infective for both lymphoid and macrophage-like cell lines. This ability to invade both types of cells was maintained through a series of passages. Furthermore, antigens, prepared from both lymphoblastoid cell lines and monolayer (macrophage-like) cultures and tested in the indirect fluorescent antibody test against various antisera, had similar reactions. Although the presence of more than one parasite could not be disproved, these results suggested to Grootenhuis (1979) different behavioural pathways within the host rather than the involvement of different parasites.

Grootenhuis *et al.* (1979) concluded that the *Theileria* of eland studied by them usually behaved like a *Theileria*, but occasionally like a *Cytiauxzoon*. On the weight of the evidence of these findings they suggested that *Cytiauxzoon taurotragi* (Martin & Brocklesby, 1960) should be named *Theileria taurotragi*. Basing his conclusion on the findings on *T. taurotragi*, Brocklesby (1979) also agreed that the name *Cytiauxzoon* should be dropped in favour of the name *Theileria*. For this reason the theilerial parasite seen in the present study in sable antelopes was identified as a *Theileria* sp., despite the fact that the criteria of the genus *Cytiauxzoon* were met.

The potential importance of the *Theileria* in sable was not determined in this study, but *Theileria* (=Cytiauxzoon) spp. have been found to cause mortalities in the common duiker, *Sylvicapra grimmia* (Linnaeus, 1758), by Neitz & Thomas (1948); in the greater kudu, *Tragelaphus strepsiceros* (Pallas, 1766), by Neitz (1957); in eland, *Taurotragus oryx pattersonianus* Lydekker, 1906, by Brocklesby (1962); in giraffe, *Giraffa camelopardalis* (Linnaeus, 1758), by McCully, Keep & Basson (1970); in roan antelope *Hippotragus equinus* (Desmarest, 1804), by Wilson *et al.* (1974); and in tsessebe, *Damaliscus lunatus* (Burchell, 1823), by Basson, Bartsch, Bigalke, Wilson & Thomas (unpublished observations, 1974).

### *Anaplasma*

In the past it has been shown that *Anaplasma* spp. of domestic animals can be transmitted to various African antelopes. *A. marginale* has been transmitted to blesbok, *Damaliscus dorcas* (Pallas, 1766) and duiker, and *A. centrale* to blesbok (Neitz & Du Toit, 1932). *A. marginale* has also been transmitted to black wildebeest, *Connochaetes gnu* (Zimmerman, 1780) and *A. ovis* to blesbok (Neitz, 1935; 1939). Peirce (1972) found that *A. marginale* occurred in eland in Kenya. Sera of antelope species living on farm land where cattle are dipped frequently showed fewer positive reactions in the capillary tube agglutination and indirect fluorescent antibody tests against *A. marginale* than sera of antelope grazing in the vicinity of non-dipped cattle (Löhr *et al.*, 1974). It has consequently been suggested that antelope species in South Africa may be the natural hosts for the *Anaplasma* sp. of domestic ruminants (Neitz & Du Toit, 1932).

However, it must also be noted that game animals do harbour other *Anaplasma* spp. Löhr & Meyer (1973) showed that wildebeest, *Connochaetes taurinus* (Burchell, 1823), Coke's hartebeest, *Alcephalus buselaphus cokii* Günther, 1884 and Thomson's gazelle, *Gazella thomsoni* Günther, 1884, in Kenya harboured *Anaplasma* spp. which could be transmitted to cattle but did not protect the cattle against challenge with *A. marginale*.

The present study would seem to add to the evidence that there are *Anaplasma* spp. or strains distinct from those normally harboured by domestic cattle and sheep. The organism in sable antelope morphologically resembled *A. marginale* yet was not infective to cattle. It did result, however, in the production of antibodies in a sheep and a few marginal bodies were seen in the blood smears.

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