INVESTIGATIONS ON THE DUIKER *(SYLVICAPRA GRIMMIA)* AND ITS BLOOD PROTOZOA IN CENTRAL AFRICA*

By I. F. KEYMER†

London School of Hygiene & Tropical Medicine

(*Communicated by P. C. C. Garnham, F.R.S.—Received 16 January 1968—Revised 14 June 1968*)

[Plates 26 to 31]

CONTENTS

* Most of this publication was presented in partial fulfilment of the requirements for the Doctor of Philosophy Degree in Zoology at the University of London.

† Present address Zoological Society of London, Regent's Park, London, N.W.1.

Vol. 255. B. 798. (Price £2 12s.; U.S. $6.75) 5 [Published 27 February 1969

The research is based on material collected from 110 duikers (*Sylvicapra grimmia*) in Malawi, Rhodesia and Zambia.

The habitats of the duiker in Central Africa are described and also various other aspects of ecology. The feeding and management of the animal is discussed, fostering youngsters on to goats being found more successful than rearing on the bottle.

The technique of splenectomy is described. Acepromazine was used for sedation and intravenous thiopentone sodium for anaesthesia, the latter being maintained with ether when necessary.

Plasmodium (*Vinckeia*) *brucei* and *P.* (*V.*) *cephalophi* were rediscovered in Malawi.

P. (*V.*) *brucei* is described in detail from blood films and compared with *P.* (*V.*) *cephalophi*. The organism was transmitted to another duiker by blood inoculations. The course of the blood infection in a naturally infected splenectomized antelope is described. Attempts to find the vector and exoerythrocytic stages were unsuccessful.

Theileri-like trypanosomes, i.e. *Trypanosoma* (*Megatrypanum*) spp.—were frequently found in duikers. The parasites have been recorded from at least 23 different species of ruminants. The organisms from duikers are easily cultured on artificial media but difficult to maintain. Attempts at transmission by blood inoculation produced inconclusive results and the vector of the duiker parasite was not discovered. It is possible that the duiker is the host of more than one species of *Trypanosoma* (*Megatrypanum*). Detailed morphological studies, however, of the trypanosomes using biometrical methods failed to reveal any clear-cut statistical difference between the parasites in different duikers from different localities or between the parasites from duikers, cattle and other ruminants. It is considered preferable at this stage not to include *T* (*M.*) *ingens*, *T* (*M.*) *tragelaphi* and *T.* (*M.*) *cephalophi* in the synonymy of the older species *T* (*M.*) *theileri*, although they may conveniently be referred to as '*theileri*-like trypanosomes'.

A brief description of other trypanosomes found in the duiker is given and other records are mentioned.

Just under 2% of the duikers in this survey were found to be infected with piroplasms, probably *Cytauxzoon sylvicaprae*. A detailed description of the organisms is given.

Several duikers in Rhodesia and Malawi were found infected with *Sarcocystis*. The duiker appears to be a new host record for this parasite. The spores of the organism, as seen in blood films, presumably released from ruptured cysts, are described. The parasite may be a new species of *Sarcocystis*.

Introduction

The late Sir David Bruce is famous for a variety of contributions to medical and veterinary science which he made during the early part of this century. Those of special interest to protozoologists include his discovery of two species of *Plasmodium* parasites in the duiker (*Sylvicapra grimmia*) and descriptions of new species of large trypanosomes in this antelope and the bushbuck (*Tragelaphus scriptus*) under the names of *Trypanosoma cephalophi* and *T. ingens* respectively. The descriptions of all these parasites were published in the *Proceedings of The Royal Society*, series B, half a century ago (Bruce *et al.* 1909*a*, *b*, 1913*a*, *b*, 1915).

The original work on pathogenic trypanosomes of man and domestic animals which Bruce and his colleagues carried out whilst working for the Sleeping Sickness Commission in Nyasaland (Malawi) and other areas of Africa has been followed up by other research workers in tremendous detail during the last 50 years but the malaria parasites of the

duiker have virtually defied all attempts at rediscovery. The validity of *T. cephalophi* and *T. ingens* as true species has frequently been questioned, owing to their close similarity to *T. theileri*, which was also originally described by Bruce and named by him after Theiler (Bruce 1902).

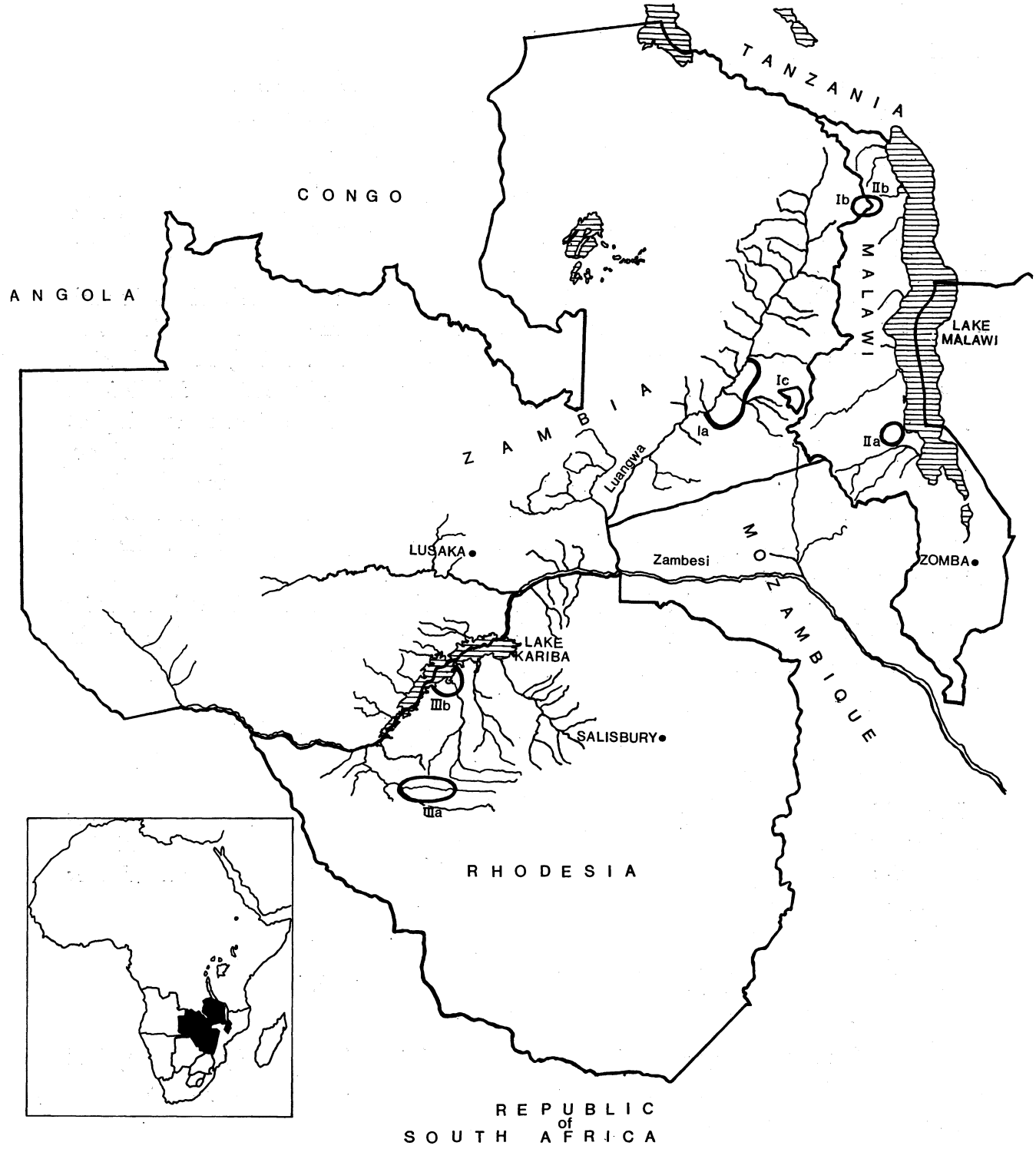


FIGURE 1. Map of Africa showing areas of study in Zambia, Malawi and Rhodesia.

Plasmodium (*Vinckeia*) *cephalophi* which Bruce discovered in Nyasaland (Bruce *et al.* 1913*a*, *b*) was not reported again until it was rediscovered in the same district and also in Rhodesia in 1964 by Keymer (1966). This organism as originally described was remarkable and different from all other known mammalian *Plasmodium* species in the enormous size of the erythrocytic schizonts and merozoites. These peculiarities puzzled malariologists until Keymer (1966) reported the presence of different and more typical schizonts in association with gametocytes such as those described by Bruce, thus enabling the taxonomic position of the parasite to be clarified. It was during the course of these investigations that another duiker *Plasmodium* parasite, which had also been seen by Bruce was rediscovered. Bruce *et al.* (1915) did not name the organism but simply illustrated it without giving descriptions. Until its rediscovery in Malawi the parasite had only been seen by Garnham in blood smears from duikers obtained in the Congo and Tanzania. Garnham (1966) named the organism *P.* (*V.*) *brucei* and a more detailed description of the parasite from Malawi is given here.

Whilst searching for the malaria parasites of the duiker it soon became obvious that this antelope was frequently infected with *theileri*-like trypanosomes some of which were similar to those encountered by Bruce, whilst other resembled *T. theileri*, *T. tragelaphi* or were of uncertain identity. What is thought to be the largest collection of *theileri*-like trypanosomes in existence was eventually obtained, enabling detailed morphological studies by statistical methods to be carried out, the results of which are also published here.

During the course of the above-mentioned investigations other parasites were found and are described, including piroplasms and *Sarcocystis*. The latter does not appear to have been recorded previously in the duiker and it is possible therefore that this parasite may be a new species.

The protozoological investigations of the duiker inevitably necessitated detailed studies of the animal and in this paper various topics related to ecology as well as management in captivity, anaesthesia and surgery are also included.

Materials and methods

Hunting methods

In addition to keeping live duikers in captivity it was also necessary to shoot a large number of wild specimens and, as these animals are mainly nocturnal, special permission was obtained from the authorities to shoot them at night using a slow-moving Landrover and powerful torches.

Collection and preservation of specimens

All animals which had been shot were weighed and measured.

Blood smears

Several thick and thin smears were made from all duikers immediately after they had been shot, the blood being taken from the heart with a sterile disposable syringe.

Examination of fresh blood

(*a*) *Trypanosomes.* Fresh blood was only examined in the field when attempting isolation and transmission of *theileri*-like trypanosomes. As soon as blood smears had been made

from the shot animal, about 20 ml. of heparinized blood was immediately centrifuged for about 15 min using a hand-centrifuge attached to a suitable part of the Landrover. About 9 ml. of blood from each tube were then discarded and the deposit used for immediate injection into experimental animals. A drop of this centrifuged blood was also examined on the spot with a portable binocular field microscope using a torch as a source of light, as these investigations were always carried out at night. Although sometimes it was necessary to examine the blood for as long as 10 min before finding the trypanosomes, this method undoubtedly concentrated the parasites. It was noted, however, that these large trypanosomes in blood smears made from centrifuged blood were less suitable for morphological studies than those in direct blood smears. They stained less satisfactorily with Giemsa's stain and sometimes showed signs of damage. Whether this was caused by the centrifugation or the presence of the heparin, it is not known.

Hand centrifugation is exceedingly tiring and has a number of disadvantages. If this work was repeated an electric centrifuge, generated from the Landrover, would be used.

(*b*) *Plasmodium* spp. Fresh blood from splenectomized duikers infected with *Plasmodium* spp. was regularly examined for exflagellation throughout the period of infection. A drop of blood was examined beneath a coverslip on a slide at frequent intervals, being kept moist in a Petri dish containing damp blotting paper.

Measurements of blood parasites

Measurements were made in the ordinary way with the aid of a slide micrometer, trypanosomes being measured according to the method now generally accepted and first described by Bruce *et al.* (1909*a*). The parasites were drawn in outline, including the kinetoplast, nucleus and undulating membrane and the distance measured along the midline of the parasite from the posterior end to the middle of the kinetoplast (*PK*), the middle of the kinetoplast to the middle of the nucleus (*KN*), the middle of the nucleus to the anterior end (*NA*), the anterior end to the end of the free flagellum (*F*) and the width (*W*) the latter being the broadest part of the body excluding the undulating membrane. The length and breadth of the nucleus were also measured, the length being an imaginary straight line passing through the middle of the nucleus parallel with the long axis of the body of the trypanosome. The width was measured along an imaginary line passing through the middle of the nucleus at right angles to the long axis. Distances between the posterior end and the middle of the nucleus (*PN*), the ratio of the distances *PN* to *KN*, and the total length (*L*) of the trypanosome were calculated from the measurements which had been made.

Methods of infecting experimental animals with protozoa

By injection. When attempting isolation of trypanosomes, rats and mice were injected intraperitoneally, and a calf, goats and duikers intramuscularly. On occasions, as for the attempted transmission of *Plasmodium* to ruminants, the intravenous route was also used, utilizing the radial vein.

By natural exposure to vectors. Two species of *Plasmodium* were isolated in this way by exposing splenectomized duikers to natural vectors in the bush. In order to protect the animals from predators and to facilitate the collection of mosquitoes, it was necessary to construct special houses (figure 2, plate 26) in which to keep the duikers. Three of these

houses were built in different localities and constructed of timber and 'elephant grass' of the kind used by Africans for the roofs of their houses. The walls of these huts, which were approximately 12 ft. × 12 ft. (3·7 m × 3·7 m) square, were lined with mud to facilitate the collection of resting mosquitoes after they had fed. Each hut was surrounded by a palisade of wooden poles at least 10 ft. (3 m) high, to deter predators, and the two diagonal corners of each hut had openings 3 ft. (90 cm) wide extending from the ground to the roof, whilst beneath the overhanging eaves of grass a space of about 1 ft. (30·5 cm) was left between the wall and the roof, thus facilitating the entry of mosquitoes and other winged insects. Four animals could be kept in one house, because each was divided into four pens by constructing walls in the form of a cross about 4 ft. (120 cm) high in the middle of the hut with a gap of about 1½ ft. (45 cm) between the end of the walls and the main wall of the house, for easy access.

Collection of mosquitoes, louse-flies and biting flies

Wild *Anopheles* mosquitoes were collected in the duiker pens at 2 h intervals throughout the day and night. They were caught with 'sucking tubes' as they rested on the mud-lined interior walls of the houses that were specially constructed for housing duikers and a bushbuck. The species collected included *A. gambiae*, *A. pretoriensis*, *A. coustani* and *A. rufipes* as well as *Culex* species. *Anopheles* mosquitoes which had obviously fed on blood were eventually dissected, whilst those that had not received a blood meal and *Culex* spp. were killed and preserved by pinning in the usual manner, for later identification.

Louse-flies (Hippoboscidae) were obtained by carefully searching the skin of all wild duikers as soon as they were shot. A further examination was usually made after the animal had been skinned, and whenever an extensive search was carried out the insects were always found.

Biting flies and mosquitoes were collected in the Chitala area of Malawi, no tsetse flies now being present. Only flies of the genera *Tabanus* and *Haematopota* were found. The insects were obtained by using an entomological catching-net, whilst many were caught on the inside of the windscreen of the Landrover whilst driving slowly through the bush. A representative collection of flies were not dissected, but preserved by pinning for subsequent proper identification.

Artificial feeding of mosquitoes on duikers

Wild *Anopheles* mosquitoes were artificially fed on the splenectomized duiker which became infected with *P.* (*V.*) *brucei*.

The insects were induced to feed on the animals in the usual way, by holding an inverted small jar containing them against a shaved area of the duiker's body, the splenectomy operation site being suitable for this purpose. Both duikers had been handled considerably by the time these experiments were made and had become very tame so that no sedation was necessary. The mosquitoes fed through perforations in the muslin fixed over the open end of the jar and various species fed quite readily on the duiker in this way.

Maintenance of mosquitoes and louse-flies in captivity

No special methods were employed. Mosquitoes survived quite well after they had received a blood meal and were maintained for up to 15 days by feeding soaked raisins and sultanas.

It was found that if hippoboscids were removed from duikers shot at night and left in test-tubes with cotton-wool plugs, they were always dead by the time they were needed for dissection the next day. As it was essential that they should be dissected immediately after death, they were kept alive by placing them on the external surface of the skin after this had been removed from the animal. The skin was then rolled up in a sheet of Polythene with the hairy side inwards. Under these conditions the insects remained alive for at least 12 h.

Dissection and examination of insect vectors

No special techniques were devised. The salivary glands of over 300 *Anopheles* spp. of wild-caught mosquitoes were dissected and examined under a dissecting microscope for the presence of *Plasmodium* spp. of sporozoites and the mid-guts for oocysts. Fifty-three mosquitoes which had been artificially fed on infected duikers were fixed without dissection in Carnoy's fluid as soon as they died or after they had been killed with ether, on the 11th, 12th and 15th days after feeding. Following fixation they were transferred to, and preserved in, 70 % ethyl alcohol.

A total of 79 *Tabanus* spp. and 62 *Haematopota* spp. of biting flies and 132 hippoboscids (*Echestypus* sp.) were dissected. The mid-guts and hind guts were examined for metacyclic trypanosomes. The remains of the hippoboscids were preserved in 70 % ethyl alcohol for subsequent detailed examination and identification.

The common duiker (*Sylvicapra grimmia*) Linnaeus, 1758

Vernacular name in Central Africa: Gwapi (Nyanja language) and Hisa.

Many species of game now only survive in large numbers within the boundaries of game reserves, but the duiker is still a common and widespread animal in Africa in spite of the spread of civilization. It has proved to be an exceptionally successful and adaptable species, and even in areas where ruthless game destruction has been carried out under tsetse-control operations it not only succeeds in surviving, but the population appears to increase (Child & Wilson 1964*a*).

In spite of its abundance, and its ability to survive in areas relatively highly populated by man, no special study has been made of the diseases of the duiker, although Roth & Dalchow (1957) have studied its helminth parasites in Rhodesia and Zambia and have also published the identifications of helminths collected during the present investigations in Malawi by Keymer.

Having decided to make a special study of the blood protozoa of this antelope an effort was also made to obtain as much information as possible about the ecology of the species. During the course of the investigations a total of 110 duikers was examined for blood protozoa, the antelopes being obtained from different areas as classified below, namely 1 from habitat I*a*, 3 from I*b*, 20 I*c*, 33 II*a*, 4 II*b*, 41 III*a* and 8 from III*b* (figure 1).

Geographical range and habitat

The duiker is confined to Africa, occurring throughout much of the area south of the Sahara desert from the Cape to Ethiopa, and as far west as Nigeria and Senegal (Morris 1965).

In Zambia and most parts of the continent it is found mainly in woodlands, especially

in the vicinity of flood plains, marginally in forests and often in areas of cultivation (Ansell 1960). It does not occur in dense equatorial forests, although Shortridge (1934), Stevenson-Hamilton (1947) and Best *et al.* (1962) stressed that it appreciates cover, usually being found within easy reach of patches of bush or long grass. It occurs at all altitudes almost up to the snow line. On the Nyika plateau in Zambia and Malawi, in contrast to its usual behaviour, it was often seen in broad daylight on open grassland away from cover. Whether this was due to the lack of human disturbance, scarcity of predators or the lower daytime temperatures at high altitudes which do not necessitate resting in the shade, it is difficult to say.

The animal was common everywhere in the areas covered during these studies except in the Luangwa valley of Zambia.

Physiognomy of the study area

The map (figure 1) shows the six main areas in the Central African countries of Zambia, Malawi and Rhodesia in which the present work was carried out. The countries formerly formed the Federation of Rhodesia and Nyasaland.

The various areas are described in some detail because in order to understand the epidemiology of disease and see the duiker, other mammalian hosts and the invertebrate vectors in proper perspective, a general knowledge of the ecology of the habitat is essential.

Hoogstraal (1956) very rightly drew attention to the extraordinary number of zoological inaccuracies encountered in medical and veterinary publications dealing with the epidemiology of disease. He stressed the importance of investigating a considerable segment of any fauna as an interrelated unit and the folly of either ignoring the ecology or simply concentrating on certain interesting component parts of the fauna.

The duiker habitats are described below and given the designations I*a*, I*b*, II*a*, etc. For the sake of brevity, they are referred to in this way throughout the remainder of this publication.

Habitat I—Zambia

The climate of the country is subtropical with the mean temperature ranging from 21·1 °C (70 °F) to 32·2 °C (90 °F), reaching a maximum of 39·4 °C (103 °F). The dry season extends from the end of April to early November, during which period no rain falls, the highest temperature of the year being reached in October just before the rains commence. The mean annual rainfall is in the region of 89 cm (35 in.).

(*a*) *Luangwa valley.* Camps were established at the following localities on the banks of the river Luangwa at an altitude of approximately 600 m (2000 ft.): camp no. 1, 20·9 km (13 miles) north-east of Lusangazi Game Camp on the east bank (13° 21′ S, 31° 41′ E); camp no. 2, 0·8 km ($\frac{1}{2}$ mile) south of Chibembe pontoon, east bank (12° 46′ S, 32° 05′ E); camp no. 3, Mfue west bank (13° 5′ S, 31° 45′ E).

The whole area is still teeming with wild game and has been less disturbed by human activity than almost any other part of Central Africa. Numerous species of large and small animals including antelopes are present but in 1962 during a period of 3 months' intensive hunting only four duikers were shot (Ingles 1965). The mammalian fauna and habitat have been described by Ansell (1960) and Ingles (1965), whilst Keymer (1963) made a

survey of the blood protozoa of many of the mammals collected by Ingles, the results of which will be published in detail elsewhere. The human population is very small and villages of primitive Africans are well scattered in the area lying to the east of the river. The land to the west between the river and the Muchinga escarpment is completely uninhabited and forms the Luangwa Valley (South) Game Reserve. Tsetse flies of the species *Glossina morsitans* and *G. pallidipes* were prevalent in the region of all three camps and sleeping sickness was endemic in the valley, whilst cattle were completely absent owing to the presence of trypanosomiasis. Mosquitoes (*Anopheles* spp.) were also present.

The temperature in the valley is rather higher than the average for the whole country, being at a lower altitude than many other places, and temperatures sometimes rise above 37·78 °C (100 °F) towards the end of the dry season.

(*b*) *Nyika plateau.* The Zambian Government Rest House was used as the base for work in this area. It is situated at 10° 34′ S, 33° 44′ E at an altitude of 2225 m (7300 ft.).

The area covered was within 0·8 km ($\frac{1}{2}$ mile) of the Rest House and also in the Chowo Forest at 10° 36′ S, 33° 40′ E, which is near the Malawi border and at an altitude similar to that of the Rest House. This forest, although it is the largest on the plateau, covers an area of probably less than 2·86 km^2 (1 sq. mile).

The above localities are on the fringe of the plateau, which extends eastwards to the edge of the Rift Valley and covers a virtually uninhabited area of approximately 2574 km^2 (900 sq. miles), ranging in altitude from about 2100 to a little over 2400 m (7000 to 8000 ft.). The only small centres of population are at the Rest House and at the Malawi Government Forestry Department situated approximately 16 km (10 miles) to the east, near which are relatively small plantations of introduced conifers. Both communities consist of a few families of Africans who cultivate small plots of land. Most of the plateau is in Malawi and the international border lies close to the Zambian Government Rest House. On both sides of the border the area has been proclaimed a game reserve by the Malawi and Zambian governments.

The game population is widespread, and considering the vast area available the numbers of animals present seem to be small. The duiker appeared to be reasonably common and a large herd of eland (*T. oryx*) was present, numbering in the region of 100 animals. The Crawshay's zebra (*E. burchelli crawshayi*) was also common. Other game animals occurring in smaller numbers included roan antelope (*Hippotragus equinus*) and reedbuck (*Redunca arundinum*). The commonest of the larger predators was the side-striped jackal (*Canis adustus*), whilst leopards (*Panthera pardus*) and lions (*P. leo*) occurred in small numbers.

The tsetse fly (*Glossina* spp.) is absent from the area and there also appear to be no *Anopheles* species of mosquitoes.

According to Brass (1953) the principal soils of the area are red or reddish loams. The plateau consists of vast undulating savannah grasslands with remnant patches of dense montane rain-forest occupying some of the valleys and gullies on either side of the streams which drain the high plateaux. These predominantly broad-leaved evergreen forests are larger and more prevalent on the eastern side in Zambia than in other areas, although there are a few fairly large patches on the western escarpment in Malawi. In spite of efforts by both countries to control burning of the grasslands, most of these magnificent forests are slowly being engulfed, year by year as the fires advance. It is generally believed that

burning has been practised by Africans on the plateau for a considerable time, in order to produce a fresh growth of grass and attract game. Indeed, Brass (1953) considered that fire is probably the chief agent of deforestation on the plateau.

The actual grasslands are completely treeless except where rocky outcrops occur, providing sufficient shelter for small stunted shrubs. The montane forests and grasslands contain a wide variety of plants and these have been listed by Brass (1953).

The climate of the area is temperate owing to the high altitude. The mean annual rainfall is probably about 150 cm (60 in.) on the western side of the plateau (Cater 1954) and the mean annual temperature below 18·3 °C (65 °F). The eastern portion in Zambia, however, is drier than the western part. In the cool months of the dry season during June and July the air temperature may fall as low as 4·4 °C (40 °F) and ground frost occurs at night. Although most of the rain falls between November and April, showers may occur at almost any time and even at the height of the dry season in late September and October. Sometimes, especially during the months of June and July, the south-east trade winds bring spells of mist and cold rain which are referred to as 'Chiperonis' and are confined to Nyika and other high plateaux on either side of the Rift Valley.

(*c*) *Chipangali and Kalichero areas.* A camp was established for only a few days at Kalikali, most of the specimens obtained in this district being kindly provided by Mr V. J. Wilson of the Veterinary Department. The area covered by him during tsetse fly control operations extended approximately from 13° 5′ S to 13° 25′ S and 32° 25′ E to 33° E. This area is situated about 78·3 km (30 miles) north of Fort Jameson and according to Wilson (1965) covers an area of approximately 572 km^2 (200 sq. miles) and is at an altitude between 760 and 900 m (2500 and 3000 ft.).

Part of the area is populated by Africans engaged in primitive methods of cultivation and cattle raising, but most of the district is uninhabited, and prior to tsetse control operations apparently supported reasonably high populations of game animals, including duiker, greater kudu (*Tragelaphus strepsiceros*), bushbuck (*T. scriptus*), roan and sable antelopes (*Hippotragus niger*). Indeed during 1963 following a halt of 2 years after a 12-year period of incessant hunting (Child & Wilson 1964*a*) duikers were still common.

Although *Glossina* species of tsetse flies have been virtually eradicated from the area, *Anopheles* species of mosquitoes are prevalent.

The Chipangali and Kalichero districts are contiguous and have been described by Child & Wilson (1964*b*).

According to Wilson (1965) the mean annual rainfall is between 76 and 104 cm (30 and 40 in.), although as much as 150 cm (59 in.) has been recorded. The mean maximum temperatures range from 27·8 °C (82 °F) to 30 °C (86 °F) and the mean minimum temperatures from 12·2° C (54 °F) to 16·7 °C (62 °F).

Habitat II—Malawi

The climate of Malawi is similar to that of Zambia, with the same distinct hot dry season and relatively cool rainy season, the mean annual temperatures and rainfall figures falling within the same range.

(*a*) *Chitala area.* This is the district in which most of the work on duiker parasites was carried out.

The base was situated at the Chitala Experimental Station of the Ministry of Agriculture 600 m (2000 ft.) above sea level, at 13° 41′ S, 34° 16′ E and about 20·9 km (13 miles) from the shore of Lake Malawi (formerly known as Lake Nyasa).

The area in which duikers were examined extended from 13° 40′ to 13° 44′ S and from 34° 9′ to 34° 14′ E at an altitude ranging from 600 m (2000 ft.) to 900 m (3000 ft.).

Camps were established within this area in the bush at three sites and in altitude they ranged from approximately 700 m (2300 ft.) at camp no. 2 to 820 m (2700 ft.) at camp no. 1.

Hunting and vector investigations were carried out within a mainly uninhabited area of hilly and undulating country about 57·2 km^2 (20 sq. miles) in extent and above 760 m (2200 ft.) which formed part of a larger area of open-type mixed woodland containing several species of trees, representing about a dozen main genera, *Brachystegia* and *Isoberlinia* species predominating. Below this altitude in the vicinity of the base camp the original vegetation was similar, except in the vicinity of the Chitala river where riverine thicket is fairly dense. This area extending to the shore of Lake Malawi was thickly populated with Africans engaged in mainly primitive methods of agriculture. Large numbers of goats were kept and mainly in the vicinity of Chitala the uncultivated areas were grazed by cattle. Shifting cultivation of maize and other crops with continual bush clearing was practised and the areas under cultivation will probably spread rapidly into the less fertile uplands as the human population increases. There is little doubt that the duiker habitat, which in 1965 was only sparsely populated by humans and appeared to support a fairly high density of these animals, will be virtually destroyed within the next decade. In spite of the disturbance, however, a few duikers and even bushbuck were present in the Chitala area, but the latter were absent from the drier uplands, where the duikers were much commoner and found associated with an apparently smaller population of Sharpe's grysbok (*Rhaphicerus sharpei*). Other game seemed to be normally absent from the area, but large troops of baboons (*Papio* (*Chaeropithecus*)) sp. were seen in the uplands and vervet monkeys (*Cercopithecus aethiops*) and bush-pigs (*Potomochoerus porcus*) were common in the vicinity of maize gardens and other cultivated ground. Predators were represented by a few spotted hyaenas (*Crocuta crocuta*), leopards and the occasional lion, genet (*Genetta* sp.) and civet (*Viverra civetta*), whilst on one occasion even a pack of wild dogs (*Lycaon pictus*) was seen.

The types of vectors of blood protozoa present in this area are discussed in detail later.

(*b*) *Rumpi–Mutunaira area.* This was by far the most densely populated area in which animals were collected, a camp being established in the middle of the African village of Mutunaira. This village is situated at approximately 11° 7′ S and 33° 35′ E at an altitude of about 1220 m (4000 ft.), approximately 24 km (15 miles) west of Rumpi and a similar distance east of the Vwaza marsh.

Much of the land was primitively cultivated, maize being the chief crop. Scattered banana trees were grown in the villages, which were almost contiguous in places. The centres of population were separated by patches or belts of 'bush' in which *Brachystegia* spp. and *Combretum* sp. predominated. The terrain is not unlike the Kalichero district of Zambia, with fairly fertile soil and a few rocky hills about 60 m (200 ft.) rising from the rather flat country around. In spite of the high human population and the presence of

village dogs, duiker and Sharpe's grysbok seemed to be quite common, especially the former, and smaller numbers of reedbuck were also present. Duikers were shot within a radius of 8 km (5 miles) of the camp.

Anopheles species of mosquitoes were present but the tsetse fly (*Glossina* spp.) had been eradicated.

It has not been possible to obtain any detailed information concerning the climate of the district. Owing to its fairly close proximity to the Nyika Plateau, the annual rainfall, however, is probably a little higher than the average for the country.

Habitat III—Rhodesia

The climate of Rhodesia is more varied than that of either Zambia or Malawi. The year is also divided into a wet and a dry season, although in parts of the south and west near the Kalahari desert it is not uncommon for the annual rains to be very low or even fail completely.

Zambezi valley

(*a*) *Nagupande—Cewali area.* The duiker material collected in this district was made available through the kindness of Dr H. Roth with the permission of the Director of the Department of Veterinary Services. The Cewali and Nagupande areas are both in the Binga district and are contiguous, lying approximately 145 km (90 miles) due south of the Zambezi river and Kariba Lake. The area is at the edge of a *Glossina morsitans* fly belt and *Anopheles* species of mosquito were present. Numerous species of game animals, of a variety almost as great as that occurring in the Luangwa valley in Zambia, were shot during tsetse control operations. The Department set up two main camps, one in the Cewali area at 18° 30′ S, 27° 45′ E and the other in the Nagupande river area at 18° 10′ S and 27° 40′ E. The Nagupande area covered an area of approximately 572 km^2 (200 sq. miles) at an altitude of around 1140 m (3750 ft.) above sea level.

Both areas were only thinly populated and the Nagupande area has been described by Child & Wilson (1964*b*).

The mean annual rainfall for this area is between 63·5 and 76·2 cm (25 and 30 in.) and the mean annual temperature probably similar to that of the Luangwa valley in Zambia, although it has not been possible to obtain any accurate information regarding this subject.

(*b*) *Kariba area.* This lies about 145 km (90 miles) north of the previous habitat and is now occupied by the Kariba dam. Blood smears taken from eight duikers which were rescued during the well-known Kariba Animal Rescue Operation was the only material examined from this area.

Description and behaviour

Eight subspecies were recognized by Ellerman, Morrison-Scott & Hayman (1953) and they also listed numerous synonyms. The subspecies occurring in this study area, however, are not well defined, and the only one listed by them is *S. g. splendidula* Gray, 1871, in Matabeleland, Rhodesia (i.e. habitat III*a* and *b*). Ansell (1960) stated that this race occurs in Zambia, but considered that it was replaced in the Eastern Province (i.e. habitats I*a*, *b* and *c*) by *S. g. orbicularis* Peters, 1852, which according to him is the Malawian form (habitats II*a* and *c*).

Detailed descriptions of the animal are provided in such publications as those by Best *et al.* (1962), Roberts (1951) and Shortridge (1934), whilst Wilson & Clarke (1962) have made a special study of the body weights and measurements of duikers in Zambia.

Although some races are greyer in colour than others, the English name of 'common' duiker as used by Ansell (1960) is preferable to the usual name of 'Grey' duiker, because quite marked colour variations occur. Very young animals appear to be rather darker than adults and it was noticed that the first moult began at about 6 weeks of age. Adult males (figure 3, plate 26) have short, straight spiky horns usually 75 to 100 mm (2·5 to 4 in.) or sometimes more in length, whilst they are usually absent in females (figure 4, plate 27) or very much smaller (Shortridge 1934). Both sexes reach a height at the shoulder of about 50 to 60 cm (20 to 24 in.), the females tending to be larger than males. The females also appear to be heavier than the rams, reaching 13·6 kg (30 lb.) or more and the latter about 11·6 kg (28·5 lb.), although the weight difference is likely to be accounted for by the gravid conditions of many of the ewes when weighed (Wilson & Clarke 1962).

Prominent face glands are present in both sexes, situated anterior to and slightly below the eye, and appear as dark slits. They are most active in males and exude a black watery secretion, which is said to be rubbed on to twigs and branches to mark territory.

The shyness and alertness of the animal is well known, and it was interesting to note that even the hand-reared lambs obtained shortly after birth would instantly sink to the ground at the slightest sign of danger, such as the sight of something unfamiliar or the sound of a sudden noise. They were always reluctant to cross an open area of ground and instinctively preferred long vegetation, carefully choosing a thick patch with overhead cover in which to rest.

The duiker is generally stated to be a solitary animal and in the Chitala area of Malawi and on the Nyika Plateau this certainly seemed to be the case. On a few occasions, however, in the Chitala area, pairs were observed and Wilson & Clarke (1962) reported the frequent occurrence of pairs in Zambia. Shortridge (1934) stated that the antelope may be seen either singly or in pairs.

Much needs to be learned about the breeding behaviour. Lambing occurs throughout the year, although there appear to be peak periods (Riney & Child 1960). Normally only one lamb is born at a time, although according to Shortridge (1934) twins are occasionally produced. Kenneth & Ritchie (1953) gave the gestation period as 120 days, although Morris (1965) stated that 196 days has also been recorded. Other figures were quoted by Shortridge (1934). In the female, sexual maturity appears to be reached at approximately 8 months (Ansell 1963). The reproductive rate in a heavily hunted area studied by Child & Wilson (1964*a*) was thought to be unusually high, because in addition to shooting a high proportion of juveniles they observed an apparently pregnant female with a lamb 'at foot', which judging by its size was considered to be less than 1 month old. The uteri of three lactating females shot by them also contained small foetuses. The latter phenomenon, however, was observed in the Chitala area on one occasion, where hunting had been practised on a much less intensive scale than in the Cewali district of Rhodesia in which their studies were made. Wilson & Clarke (1962) and Ansell (1960) also recorded lactating females in early pregnancy in two separate areas of Zambia, so this may well be normal and not one of the indications of an increased rate of reproduction as suggested by Child &

Wilson (1964*a*). The breeding pattern in a Tsetse Control Shooting Area of Rhodesia has also been investigated by Riney & Child (1960) by studying many skulls collected over a period of 14 months and attempting to work out the ages of animals on the basis of tooth eruption.

Studies on population and nocturnal behaviour in the Chitala area, Malawi

In the wet season during the months of January and February a count was made of all the duikers seen or collected on night hunting expeditions. An old road covering a distance of 8·8 km (5·5 miles), which had been closed to all vehicles and passed through habitat II*a* previously described, was used on every occasion. The only times that hunting was not carried out was when it was raining. Although no details of cloud cover or moonlight were recorded, the impression was obtained that these factors had no effect on the number of animals seen, contrary to the statement by Shortridge (1934) that duikers are most active on moonlight nights. The area hunted was completely uninhabited and the road was never used after nightfall by the African population, so that apart from natural predators the duikers were undisturbed. Visibility on either side of the road was fairly constant, ranging from approximately 33·6 to 89·6 m (30 to 80 yards).

In January, on 15 hunting excursions between dusk and midnight, 13 duikers were observed or collected, giving an average of 0·86 per night. A year later during January and February a figure of 0·9 per night for the same period was obtained, after 19 hunts. Between midnight and sunrise, however, the appreciably higher figure of 1·5 per night was obtained for only 12 hunts. Obviously no definite conclusions can be drawn from these observations. It would appear, however, that duikers in this area at least, are most active during the 3 h prior to sunrise, when most of the excursions after midnight were made. On only two occasions were no duikers seen during this period, whilst once a total of seven were observed within a distance of approximately 17·7 km (11 miles), i.e. 1·4 animals per mile. This figure compares with 1·5 per mile observed by Child & Wilson (1964*a*) in Rhodesia during the late afternoon. In the Malawi habitat duikers were seldom observed during the day, although admittedly no organized observations were made, and on 13 occasions out of a total of 34, between dusk and midnight no duikers were observed.

It was impossible to determine the sex ratio in this population because at night it was seldom possible to be sure of the sex of an animal until it was shot. A total of 21 duikers was collected in the area, representing 16 males, and 5 females all of which were pregnant. Ten males and 3 females were shot before midnight and 6 males and 2 females after midnight, the ratio of total rams to ewes being just over 3:1, roughly this same proportion being maintained for those collected both before and after midnight. This sex ratio is different from those of Wilson & Clarke (1962) in Zambia and Child & Wilson (1964*a*) in Rhodesia. Out of a total of 61 animals in the Rhodesian collection there were also more males than females, but in the lower proportion of 1·3 to 1, the figures being 35 and 26 respectively, whilst in Zambia the sexes were in almost equal proportions—99 males to 94 females. Whether or not the males definitely outnumbered the females in the Malawi habitat it is impossible to say, because this may well reflect a behavioural pattern. Perhaps

males, being more dominant and aggressive, are more reluctant to flee at night than females, when faced with the unusual experience of being dazzled with a bright torch beam, and are therefore more easily shot. If this is true, then it might account for the almost equal proportion of the sexes in Wilson's collection, who hunted by day.

Natural feeding habits

Until Wilson & Clarke (1962) analysed the stomach contents of nearly 200 duikers shot in Zambia there was little reliable information concerning the food preferences of the species in the wild.

These workers gave a list of the plants which were eaten throughout each month of the year, and when providing food for duikers in the present investigation full use was made of this information. Whilst walking with tame duikers on a lead in the bush a note was made of the identity of the various plants which were eaten. As is well known, the duiker is primarily a browser, and in addition to eating some of the plants recorded by Wilson & Clarke during January and February the animals were also seen to eat the bark of Cheyo (Nyanja language), bark and leaves of Cewo (N) (*Vellozia* sp.), leaves of Chitono (N), (*Commiphora mollis*), Bwazi (N) (*Securidaca longipedunculata*), Kamphori (N) (? *Brachystegia* sp.) and Chipembere (N) (*Randia buchananii*).

Records were kept of the stomach contents of the duikers which were shot, but there was no time to study the subject in detail. The most interesting findings were the presence of soil and very small stones in the rumen of many animals. Wilson & Clarke did not record this observation, probably because, except when the ruminal contents are sieved whilst searching for helminths, the presence of soil is not usually detected.

Undoubtedly some earth is eaten when food such as fallen fruits and flowers are taken from the ground, but tame duikers were also observed to deliberately eat soil. The leaves of Mombo (N) (*Brachystegia boehmii*), a plant which is used by Africans for making string, were identified in the rumen on one occasion and pieces of fungi ('toadstools') or Mbowa (N) were frequently found during January and February. Wilson & Clarke (1962) also recorded the presence of fungi during the wet season, but not in February.

Feeding and management

The feeding of adult duikers which had become used to captivity was relatively simple, because in addition to eating the leaves of various trees normally taken in the wild (Wilson & Clarke 1962), they would also readily eat whole or kibbled maize, maize meal, banana and mango peelings, mango leaves and a variety of relatively easily obtained foodstuffs. Water was usually readily accepted. Best *et al.* (1962), however, stated that the animal is capable of going without it for a long period and Lydekker (1926) pointed out that duikers may be found in waterless localities.

Feeding and rearing newly born duikers, which were obtained from African hunters, proved to be much more difficult and time-consuming than maintaining adults, because they were often received in a shocked, half-starved or injured state. At first, an attempt was made to rear these animals by bottle-feeding on cow's milk. The composition of duiker's milk appears to be unknown. On the assumption, however, that it probably resembles the milk of other antelopes such as the impala, Grant's gazelle (*Gazella granti*), Thompson's

gazelle (*G. thomsoni*) and Palestine gazelle (*G. gazella*), all of which according to Ben Shaul (1963) contain about 19 to 20% fat, a small quantity of melted butter was sometimes added to the milk. This was usually readily taken once the animal had become accustomed to the bottle and providing the milk was offered warm at approximately body temperature. For the first 2 weeks of life the lamb was fed as much as it would take (often only about half a baby's feeding bottle, i.e. 8 fluid oz. or 226·2 ml.) every 6 h throughout the day and night; feeding seldom taking less than 20 min. From 2 to 4 weeks of age feeding times were reduced to every 8 h, by which time the youngster would normally be consuming up to a pint (28·4 ml.) at each meal. From 4 weeks of age feeds were given at 12 h intervals, by which time the animal would be eating small quantities of leaves which were provided fresh, twice a day.

Although baby duikers were reared artificially in this way it was not always satisfactory and intestinal upsets occurred characterized by diarrhoea, which resulted in loss of weight and occasionally death. It was decided therefore to try fostering the babies, as soon as they were obtained, on to female goats which had recently kidded and had a good supply of milk. By keeping records of body weight, shoulder height, and length from nose to tail at approximately weekly intervals of all the lambs, it soon became obvious that fostering was a complete success, in spite of the fact that goat's milk probably has a lower fat content than that of the duiker. The babies were fed at the same periods, according to age, as those which were bottle-fed and they learned to feed from their foster parents much more quickly and eagerly than from the bottle. Unfortunately, however, none of the goats took readily to this imposition and they had to be restrained by two people to avoid their kicking or biting the baby duikers (figure 4, plate 27). It was also noticed that the young duikers showed a definite preference for goats with slender and long teats rather than those with wider stumpy ones, which they had difficulty in suckling. After suckling, the baby's hind quarters were always stroked in the region of the anus with a moist piece of toilet paper or cotton wool to simulate the action of the dam's tongue. This invariably stimulated urination and usually defaecation, thus preventing constipation. At about 5 weeks of age this artificial stimulation became unnecessary, because the youngster would by then be excreting without aid.

As soon as the baby duikers were acquired they were fitted with collars and they soon learned to walk quite well on a lead. By this means they could be exercised each day and given a chance to find some food for themselves. This also provided an opportunity to study their feeding habits. They first started showing an interest in eating bark, dead leaves, soil and the flowers and fresh leaves of various herbs at about 2 weeks of age, but no real quantity of vegetable matter was consumed until they were 3 to 4 weeks old, when they commenced ruminating. The babies also indulged in coprophagy from an early age, not only eating their own faeces but also those of the goats. Wilson & Clarke (1962) did not mention coprophagy and it does not seem to have been recorded previously in duikers or other ruminants although it is well known (Southern & Thompson 1964) in another herbivorous animal, i.e. the European rabbit (*Oryctolagus cuniculus*). The phenomenon was only observed in two lambs which were fostered on to a goat, and as both were thriving well there is no evidence that the coprophagy denoted a dietary deficiency of some kind. The most likely explanation is that the ingestion of faeces was purely accidental because

2

3

FIGURE 2. Special house for duikers in Habitat II*a*, Malawi.

FIGURE 3. Adult male common duiker (*Sylvicapra grimmia*). It was in this splenectomized antelope that *Plasmodium* (*Vinckeia*) *cephalophi* was rediscovered.

(*Facing p.* 48)

4

5

FIGURE 4. Adult female Duiker (*Sylvicapra grimmia*).

FIGURE 5. Baby duikers (*Sylvicapra grimmia*) with goat foster mother. After splenectomy, both of these antelopes became infected with *Plasmodium* (*Vinckeia*) *brucei*.

it was noticed at the time the babies first commenced to sample and eat leaves and other organic matter.

Transport

The experimental work frequently necessitated the transport of duikers by Landrover sometimes for quite considerable distances, therefore it was essential to design crates in which they could be safely carried. The problem of transporting these naturally nervous animals was solved far more easily than was at first expected. Crates were constructed to contain single duikers only. They were made of water-resistent hardboard with wooden frames. Each crate was in the form of a narrow rectangular box with a sliding door at each end, a space of about 2·5 cm (1 in.) being left between the top of the door and the top of the box. These spaces provided the only ventilation, the remainder of the box being entirely closed. The duiker was therefore in almost complete darkness and was unable to see outside, because the height of the crate was always made at least 15·2 cm (6 in.) higher than the animal's head. This also meant that the duiker would not be in a direct draught even when it was standing up. The floor of the crate was made of solid hardboard with the roughened surface on the inside, whilst a sack or a deep bedding of dried grass was always provided for comfort and to prevent the animal slipping. Each crate was designed so that it was only slightly wider than the animal, thus preventing the antelope from falling sideways or turning round. The duiker was loaded in one end and removed from the other. suitable dimensions for a crate to contain an adult animal were approximately 85 cm (33·4 in.) high, 115 cm (50 in.) long and 36 cm (14·2 in.) wide. Crates for younger animals were made proportionately smaller.

It was found that the duikers seldom stood up when the vehicle was moving and rested quietly whilst they were in darkness and unable to see their surroundings. Sedation proved to be unnecessary.

Sedation

Adult duikers, especially males, are dangerous animals because in addition to having sharp horns they possess 'razor-edged' hooves. They are also very nervous, so that sedation is essential before they can be safely handled without injury to themselves or the handler.

Acepromazine (Acetylpromazine veterinary: Boots Pure Drug Co. Ltd) was the only tranquillizer which was used, and although there was some variation in the response of different individuals it was found to be satisfactory in most cases. Usually the only effect at the dosage rate employed was to quieten the animal, making it more amenable to handling and restraint for such purposes as intra-cardiac puncture or intravenous injections, whilst it was always used prior to the administration of a general anaesthetic. Linzell (1964), however, did not recommend the related drug chlorpromazine as a pre-narcotic for goats because it accentuates the respiratory depressant action of pentobarbitone.

Acepromazine was administered intramuscularly using the injectable fluid containing 2 mg/ml. at the dosage rate of 0·5 to 1 mg/kg. The drug usually took about 1 h to have an affect, and this lasted for about 5 or 6 h. For sheep and cattle the manufacturers recommend the much smaller parenteral dose of 0·05 to 0·1 mg, but this was found to be ineffective for duikers and no toxic effects were observed with the higher dosages.

It was also found, as reported by Linzell (1964) when using chlorpromazine on goats in

conjunction with pentobarbitone sodium, that administration of acepromazine 1 h prior to pentobarbitone or thiopentone anaesthesia lowered the dose required for induction with these barbiturates.

Anaesthesia

When anaesthetizing ruminants it is advisable to avoid the use of a general anaesthetic whenever possible, owing to the dangers of ruminal tympany and regurgitation of the ruminal contents, both of which often occur when a ruminant is in the recumbent position under general anaesthesia, even if only for a short period.

Major surgery, which is frequently carried out on domestic ruminants with the animal in the standing position and using a local anaesthetic, is not possible with duikers.

Unfortunately general anaesthesia is essential before splenectomy can be performed on a nervous wild ruminant such as a duiker, even if the animal is under the effects of a tranquillizer. When full surgical facilities are available, however, the hazards can be reduced to a minimum by using gaseous anaesthetics such as halothane (Fluothane: Imperial Chemical Industries) and a closed method of anaesthesia with carbon dioxide absorption similar to that described by Linzell (1964), preceded by a barbiturate given intravenously. The use of a cuffed endotracheal tube, which prevents regurgitated ruminal contents from entering the trachea, is also essential.

Under field conditions the choice of anaesthetic was restricted, especially as only simple apparatus was available.

According to Wright & Hall (1961), when using pentobarbitone sodium in goats the margin between anaesthesia and respiratory failure is small. Linzell (1964) used pentobarbitone successfully in combination with cyclopropane or halothane but he made up his own solution of pentobarbitone sodium because the proprietary brand available to him contained 20 % propylene glycol in water. This he stated, causes haemolysis and haematuria in goats, as it also does in sheep (Potter 1958). The pentobarbitone sodium (Sagatal: May and Baker Ltd.) (containing 1 gr. or 60 mg/ml.) which was available in Malawi unfortunately also contained this substance although this was not known at the time. The approximate dosage level was assessed at the usual rate of $\frac{1}{6}$ gr./lb. (28·6 mg/kg.) body weight, anaesthesia being maintained with ether. Seven adult goats, a calf, two duikers (an adult and a youngster) and a bushbuck were anaesthetized. The amount of pentobarbitone which was required varied considerably, much less than the normal dose being sufficient, owing to premedication with acepromazine. Nevertheless, pentobarbitone was believed, in spite of the low doses used, to be mainly responsible for the death of three of the goats, the bushbuck and the young duiker. Lack of success with this barbiturate was thought at the time to be mainly due to its respiratory depressant property being accentuated by premedication with acepromazine, but later it was believed that the most important factor was probably the deleterious action of the propylene glycol.

It was decided, therefore, to use thiopentone sodium intravenously instead, in spite of the fact that this barbiturate is shorter-acting than pentobarbitone. When using thiopentone therefore, more ether was required to maintain anaesthesia, and this sometimes resulted in the additional hazard of excessive salivation. The approximate dose of thiopentone sodium (Intraval Sodium: May and Baker Ltd) was assessed at the same rate as pentobarbitone sodium, i.e. 28·6 mg/kg. ($\frac{1}{6}$ gr./lb.) body weight.

With both methods of anaesthesia, atropine sulphate was administered intramuscularly, at the dose of 0·65 mg ($\frac{1}{100}$ gr.) for an adult duiker weighing approximately 11 340 g (25 lb.) and 0·16 mg (1/400 gr.) for a youngster weighing 2495 g (5·5 lb.). Linzell (1964) pointed out that atropine has the disadvantage of dilating the pupil and therefore depriving one of a useful guide to the depth of anaesthesia. Nevertheless, this disadvantage did not seem to outweigh the risks of excessive salivation, and atropine was therefore used in low dosages in all animals in the hope that it would reduce salivation. Wright & Hall (1961) stated that atropine did not control profuse salivation in goats anaesthetized with pentobarbitone and whether or not it was effective in duikers and goats in the present experiments it is difficult to know. The salivation, however, could certainly not be described as profuse in all the anaesthetized animals, in spite of the additional use of ether.

Animals were always starved prior to anaesthesia for a minimum of 12 h for young duikers and 24 h for adult ruminants. This helped to some extent to reduce the volume of ruminal contents, especially in young animals, thereby minimizing the risk of regurgitation of ruminal fluid when under anaesthesia. It also reduced pressure of the rumen on the diaphragm and facilitated surgery by providing more space in which to work. After sedation each animal was weighed and then half the computed dose was given fairly quickly into the radial vein whilst the animal was held on its side. Within about 1 min relaxation usually occurred and then more thiopentone was slowly injected, the respirations being watched closely all the time to assess the depth of anaesthesia. The reaction to thiopentone, as with pentobarbitone, was very variable and testing the depth of anaesthesia with the aid of pedal and palpebral reflexes was not entirely reliable. As soon as the jaws were completely relaxed the animal was intubated before removing the syringe and needle from the vein. It was placed on its back with the head and neck fully extended. Intubation was usually quite difficult, and in one small duiker it was found to be impossible because of the small amount of space in the buccal cavity and the difficulty of seeing the larynx.

Four duikers (an adult and three youngsters) were anaesthetized by the above method. Death only occurred on the occasion when intubation proved to be impossible and then it was due to regurgitation and subsequent inhalation of ruminal contents.

When thiopentone and ether were used, recovery was often complete within 2 or 3 h, but when using pentobarbitone recovery was delayed for as much as 5 h after the cessation of ether anaesthesia.

Bleeding

For the purpose of making blood smears the ear vein was used in the ordinary way and no sedation was necessary. When large blood samples were required the duikers were tranquillized with acepromazine and then an hour later blood was taken by intracardiac puncture. The animal was held down with the left side uppermost, on a thick bedding of grass and sacks. Care was taken to always keep the head held down, thus making it impossible for the animal to raise its body. The forelimbs were held above the knees and the hind limbs above the hocks and in such a way that the body was fully extended. It was then comparatively easy to palpate the heart through the thoracic wall and insert a wide-bore needle with syringe attached in the usual way through an overlying intercostal space. As much as 20 ml. of blood were removed within a few seconds, without any

untoward effect. One adult duiker satisfactorily survived this procedure three times within one week.

Splenectomy

The technique of this operation has been described in cattle by Gates (1953) and is well known. Essentially the same method was used for all the ruminants, including duikers and bushbuck. A few points, however, concerning the operation in the various species are worth mentioning.

The initial incision was made on the left side, behind and as close to the last rib as possible, but not so close as to cause undue tension on the sutures when the skin wound was closed. It was also necessary to continue the incision dorsally to the edge of the psoas muscle because in duikers and some goats the spleen was frequently situated more anteriorly and dorsally than in the calf. In addition to being attached to the rumen, in duikers the spleen was usually adherent to the membranous part of the diaphragm. The organ is therefore difficult to reach unless the incision through the skin and abdominal wall is made as far forward and as dorsal as possible. When the dorsal extremity of the spleen was partly attached to the diaphragm it was found to be safer to commence tearing away, by blunt dissection, the attachment to the rumen, commencing at the posterior and working anteriorly and dorsally towards the diaphragm rather than to start at the dorsal edge. Especially in young duikers the tendinous part of the diaphragm is extremely thin and transparent, so that the lungs can be easily seen. Extreme care is necessary therefore to avoid rupturing the diaphragm. The spleen is also friable and easily ruptured.

In the ox the splenic blood vessels and nerves can usually be located and ligatured fairly easily but in duikers they usually enter the organ more anteriorly, being nearer the dorsal extremity and therefore less accessible than in the ox.

Post-operative care

As with all ruminants it is important, immediately the operation is over, to prop up and support duikers so that they are in the normal resting position with the sternum against the ground. In this position the chances of the ruminal contents being regurgitated and inhaled are reduced to the minimum. The animal should be closely watched at first and the endotracheal tube only removed when there is evidence that anaesthesia is becoming sufficiently light for damage to occur to the tube from the animal's teeth.

Observations on normal rectal temperature

On many occasions the rectal temperature was taken of both immature and adult healthy duikers. Records were only kept when the animal remained quiet, in order to avoid false readings due to excitation. No sedatives were used in case they should also interfere with the readings. Because of the nervous disposition of the duiker, what were considered to be normal readings could only be obtained from one particularly tame adult. Readings from six healthy lambs under 2 months of age, however, were obtained without much difficulty.

It was concluded that the normal rectal temperature varied from 37·2 to 39·3 °C (99 to 102·8 °F), that of immature animals being higher than that of the adult. The mean for the six lambs was 38·5 °C (101·3 °F) and for an adult male 37·4 °C (99·4 °F).

Ectoparasites

In Malawi (habitat II *a*) at least 91 % of the wild duikers which were collected harboured the hippoboscid fly *Echestypus paradoxus*. A similarly high percentage was found on the antelopes in Zambia (habitat I *c*), but unfortunately no information could be obtained regarding the ectoparasite infestations of the Rhodesian duikers.

Ticks were collected from duikers in Malawi (habitat II *a*) and Zambia (habitat I *c*), but were much less frequently seen than louse-flies and the infestations were lighter. Ticks from Malawi were all *Rhipicephalus* spp. including *R. neavei*, but in Zambia an *Ixodes* sp., *Hyalomma truncatum*, and *Amblyomma variegatum* were also found, whilst one species of *Rhipicephalus* was identified as *R. reichenowi*.

Blood protozoa

(*a*) *Family Plasmodiidae*

Plasmodium (*Vinckeia*) *brucei* Garnham, 1966

History and introduction

As stated previously, about the same time that Bruce *et al.* (1913 *a*, *b*) discovered *P.* (*V.*) *cephalophi* in 1912 they also found another *Plasmodium* parasite of the duiker in the same area of Nyasaland. Although they did not describe or name the organism, from their coloured illustrations trophozoites and schizonts are easily identifiable and possibly one macrogametocyte. This parasite, which has been named *P.* (*V.*) *brucei* by Garnham (1966), was rediscovered in the type habitat in Malawi (Nyasaland) during January 1964 in the blood of two adult male wild duikers which had been shot. About a year later it was found in the same locality in a young male, and eventually isolated in one animal by exposing three splenectomized duikers to the infection in the bush, in the same area where *P.* (*V.*) *cephalophi* was found in a similar fashion (Keymer 1966).

Unlike *P.* (*V.*) *cephalophi*, natural infections of this parasite were relatively heavy and easy to detect on microscopic examination. Complete thin blood smears from two of the three infected wild duikers were examined, 43 parasites being found in one film and 36 in the other. This is a much higher level of parasitaemia than occurred in a natural infection with *P.* (*V.*) *cephalophi*, and often even in splenectomized antelopes infected with this organism.

The parasite was only found in duikers in habitats II *a* and the three positive cases represented an infection rate of 13·6 % of 22 wild duikers which were shot in the area.

Transmission experiments

A splenectomized immature male duiker about 13 weeks of age became infected after exposure to natural infection in the bush during March. For less than a day prior to the appearance of the parasites in the blood the duiker had been kept at camp no. 1 after spending a period of 52 h at the base, where circumstantial evidence suggests it did not become infected. Prior to that period the duiker had been first exposed to natural infection at camp no. 2 for a period of 12 days. It can probably be safely assumed that infection was

not contracted at the base because exposure to mosquitoes was reduced to a minimum there by housing the duiker in a brick-built building in which insecticides were used. The building was also in an area where the wild duiker population was small and where tame splenectomized and entire duikers were exposed to natural infection outside for several weeks without becoming infected. The young splenectomized duiker before being transferred to camp no. 2 for its first visit to the bush had been housed in this same building at the base for 2 weeks after splenectomy without showing parasites in the blood. When parasites were first seen in its blood the animal had been at camp no. 1 for no more than 22 h, and as previously stated, immediately prior to that it was housed at the base for 52 h. If it is assumed therefore that the animal became infected in the bush during its second visit and not at the base, then the pre-patent period must be 22 h or less. If, on the other hand the animal became infected during its first visit to camp no. 2 and not at the base, then the pre-patent period must be at least 74 h, which is the time that elapsed between leaving camp no. 2 after the first exposure to natural vectors and the appearance of parasites in the blood during the second visit to the bush at camp no. 1. Similarly it can be calculated that the maximum length of the pre-patent period would be nearly 17 days, prior to that period the duiker having been at the base. A pre-patent period of 22 h or less is very quick and unlikely, because the shortest period so far recorded in mammalian malaria is nearly twice this length, namely 43 h for *P. berghei yoelii* in rodents (Landau & Killick-Kendrick 1966). Therefore the pre-patent period of this parasite probably lies somewhere between 74 h (just over 3 days) and 17 days. If this is the case then the duiker became infected at camp no. 2 between the 1 and 13 March—not earlier than 15 days after splenectomy.

Heparinized blood from one infected wild duiker was injected intraperitoneally into a non-splenectomized, approximately 3-month-old female duiker, but failed to infect it. Blood taken from the infected splenectomized animal on the 7th day of the infection, however, and injected intramuscularly into an entire male duiker about 7 weeks of age resulted in a parasitaemia 62 h later. No attempt was made to infect other ungulates by blood inoculation, but a sample of this heparinized blood was dispatched to London where, together with some serum from an infected wild duiker, it is deposited at the London School of Hygiene and Tropical Medicine.

Course of blood infection

As stated earlier, even natural infections do not remain as cryptic as those caused by *P.* (*V.*) *cephalophi*. Numerous schizonts were found in the blood of all three infected wild duikers and appeared in the blood of the splenectomized duiker on the 2nd day of parasitaemia, whilst in the duiker infected by blood inoculation schizonts were first seen on the 3rd day.

Examination of blood smears from the splenectomized duiker during the first 7 days of parasitaemia and made twice a day revealed an apparent schizogonic cycle of 48 h. By the 4th day the level of parasitaemia was quite high, it being possible to find as many as 11 parasites in a 10 min search using the 1/12 objective. No actual parasite counts were made as with *P.* (*V.*) *cephalophi*, but this corresponds almost exactly with the amount of time required to find the same number of parasites of that species, when the infection rate was in the ratio of 1 *Plasmodium* to 6000 erythrocytes. In the first splenectomized duiker

infected with *P.* (*V.*) *cephalophi* this was the highest rate of parasitaemia and it was reached on the 5th day, whereas with *P.* (*V.*) *brucei* the degree of blood infection never fell to a level as low as this during the subsequent 26 days of the infection, until the animal was killed on the 30th day. In the artificially infected non-splenectomized duiker the level of parasitaemia also remained relatively high throughout the 21 days of infection although it took about 7 days to reach a degree of infection in the region of one parasite to 6000 erythrocytes.

Although accurate counts of asexual and sexual forms in the blood were not made, the difference in the proportions of these forms and in those of the sexes of gametocytes was noticeably unlike that seen in *P.* (*V.*) *cephalophi.* From the 4th to the 7th day of infection in the splenectomized duiker, for example, gametocytes were greatly outnumbered by asexual forms in proportions ranging from 1:4·5 on the 4th day to 1:85 on the 7th, whilst male and female gametocytes appeared to be present in roughly equal proportions. In fact throughout the infection in both duikers, asexual forms greatly outnumbered gametocytes, although the proportions of microgametocytes to macrogametocytes did not always remain roughly equal, sometimes females being noticeably more common than males.

In wild duikers, gametocytes were outnumbered by asexual forms in the ratio of 1:35 in one animal and 1:4·3 in the other. There were insufficient numbers of gametocytes present to enable an accurate estimate to be made of the sex ratio, but the sexes appeared to be present in roughly equal proportions.

Fresh blood was regularly examined for exflagellation and this was first seen on the 16th day of infection, being observed again on the 19th day when the blood was next examined. On the 20th and 22nd days exflagellation was not seen and at that stage examinations were stopped. Unfortunately it was not possible to determine the number of microgametes which were produced, only one being seen with certainty. The small number of microgametocytes present in the blood in comparison with the other forms made examination for exflagellation a very time-consuming procedure and this is the reason for the lack of information regarding the phenomenon. The earliest that exflagellation was observed after the animal had been bled and the blood examined beneath a coverslip on a slide was 20 min.

Morphology

In fresh blood no striking morphological features were observed. The pigment, however, was very obvious and in some parasites it was constantly moving and appearing to rotate round its axis.

In Giemsa stained blood smears, trophozoites (figure 6, plate 28) somewhat resemble piroplasms, being roughly circular, measuring about 1 μm in diameter and consisting of a fairly well-defined area of reddish-staining chromatin and fainter bluish-staining cytoplasm. At this stage no pigment is discernible although by the time the parasite has doubled its dimensions small chips of pigment may become visible (3). These maturing trophozoites are very irregular in shape (3 and 3*a*). The dark reddish-stained chromatin is usually situated along one edge of the parasite and seldom in the middle. The cytoplasm stains pale blue, there often being an area present which stains very faintly, whilst sometimes small vacuoles may be present.

The irregular shape is gradually lost as the trophozoite enlarges and by the time one third of the erythrocytes is occupied, the parasite is roughly circular (4). The chromatin now becomes gradually more concentrated and stains more darkly. Pigment granules become larger and more crystalline and the glistening blackish appearance of the very small chips (3 and 3*a*) gradually gives rise to a distinct yellow colour (5*a et seq*). Forms of the signet-ring type (5*a*) were illustrated by Bruce *et al.* (1915) and are quite common, but they are not so distinct as those seen in *P.* (*V.*) *cephalophi* infections. The parasite at this stage, when about half the erythrocyte is occupied, varies considerably in appearance. The roughly circular outline is retained, but the chromatin takes on a variety of forms as the darker clumps of chromatin gradually become rounded off (5 and 6 to 9). At the same time the cytoplasm loses its pale blue coloration and tends to stain a mauve colour. The nuclei when about 6 to 10 in number often become arranged in a 'rosette' pattern (10). They stain a dark reddish purple and have blurred outlines. As more nuclei are produced the surrounding mauve-coloured cytoplasm stains more faintly. The erythrocyte is nearly filled by the time 8 to 12 nuclei have been formed (10 and 11). Rarely, however, does any change occur in the host cell up to this stage. By the time the schizont is nearly mature (12) the erythrocyte is slightly enlarged and completely occupied. When the schizont contains eight or more chromatin masses, the erythrocyte occasionally becomes appreciably enlarged and pale in colour, but this enlargement and loss of density is more frequently seen when the cell is occupied by a mature or nearly mature gametocyte. No Schüffner's dots have been seen and usually mature gametocytes occupy the entire erythrocyte (*b* and iii). Mature schizonts measure up to 8 μm in diameter and produce 16 merozoites (1), which are approximately 1 μm in diameter. Occasionally in the late stages of the infection a large number of merozoites have been seen, but this appeared to be due to a double infection of the erythrocyte.

It is not possible to recognize immature gametocytes with certainty until they reach the stage of nearly filling the erythrocyte. In the young microgametocytes (*a*) most of the

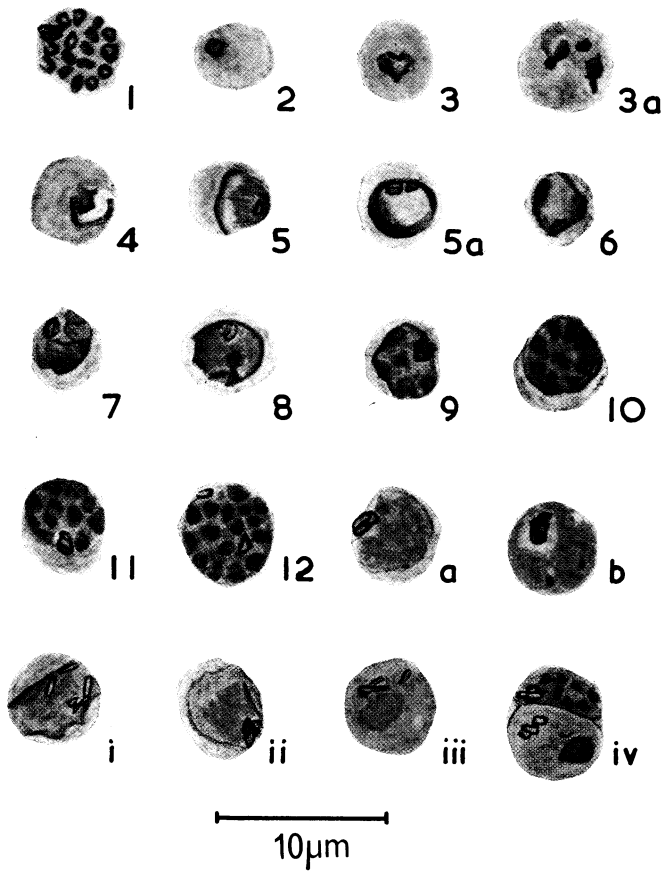
---

Description of plate 28

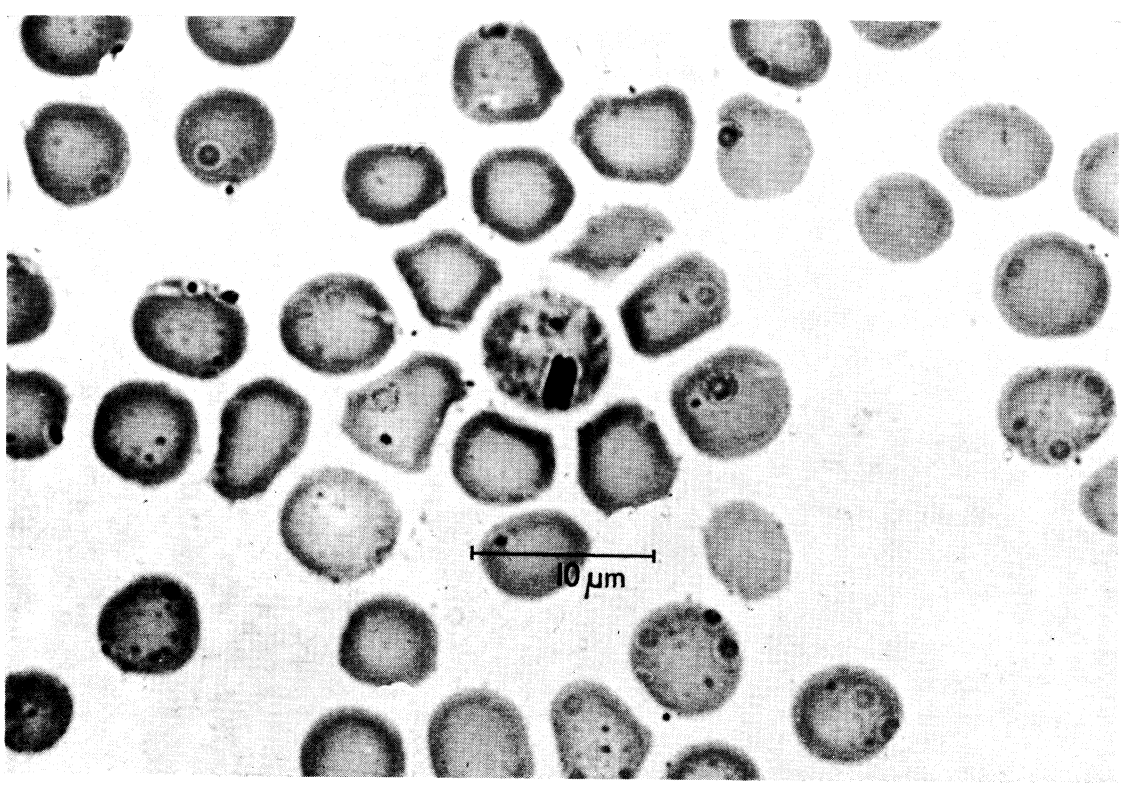
Figure 6. *Plasmodium* (*Vinckeia*) *brucei* in the blood of the duiker (*Sylvicapra grimmia*).

1	Mature schizont containing 16 merozoites		'daisy-head' or rosette arrangement of nuclei in 10
2	Young trophozoite	2	Nearly mature schizont, with two clumps of pigment
3, 3*a*	Maturing trophozoites, with a double infection in 3*a*	*a*	Immature microgametocyte
4, 5	Trophozoites	*b*	Mature microgametocyte
5*a*	Trophozoite; signet-ring type	i, ii	Immature macrogametocytes
6, 7	Trophozoites. Probably later stages	iii	Mature macrogametocyte
8, 9	Developing schizonts	iv	Erythrocyte infected with a young schizont and a macrogametocyte.
10, 11	Young schizonts; later stages. Note		

Figure 7. Photograph of a gametocyte of *Plasmodium* (*Vinckeia*) *cephalophi* in a thin blood smear from a splenectomized duiker reference no. D 1/64. Note the presence of two long and thick rectangular blocks of pigment lying side by side. This type of pigment is characteristic of *P.* (*V.*) *cephalophi*. Sometimes similar but more slender rectangular pigment granules are seen in *P.* (*V.*) *brucei*.

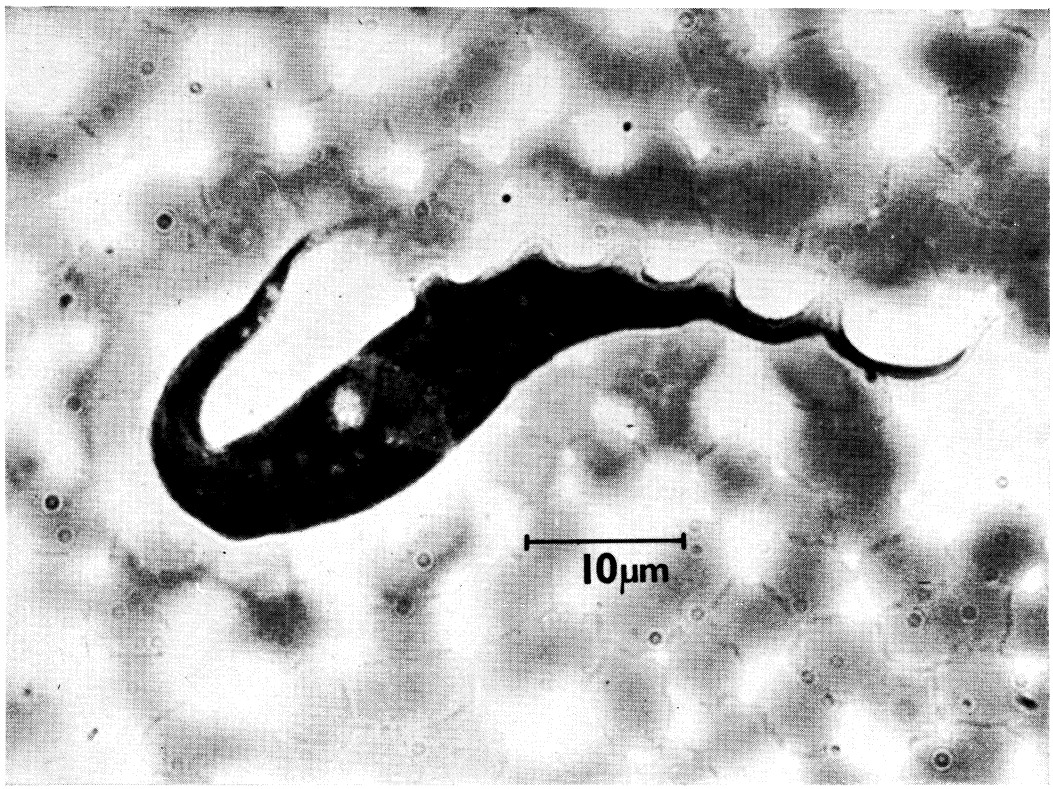


6

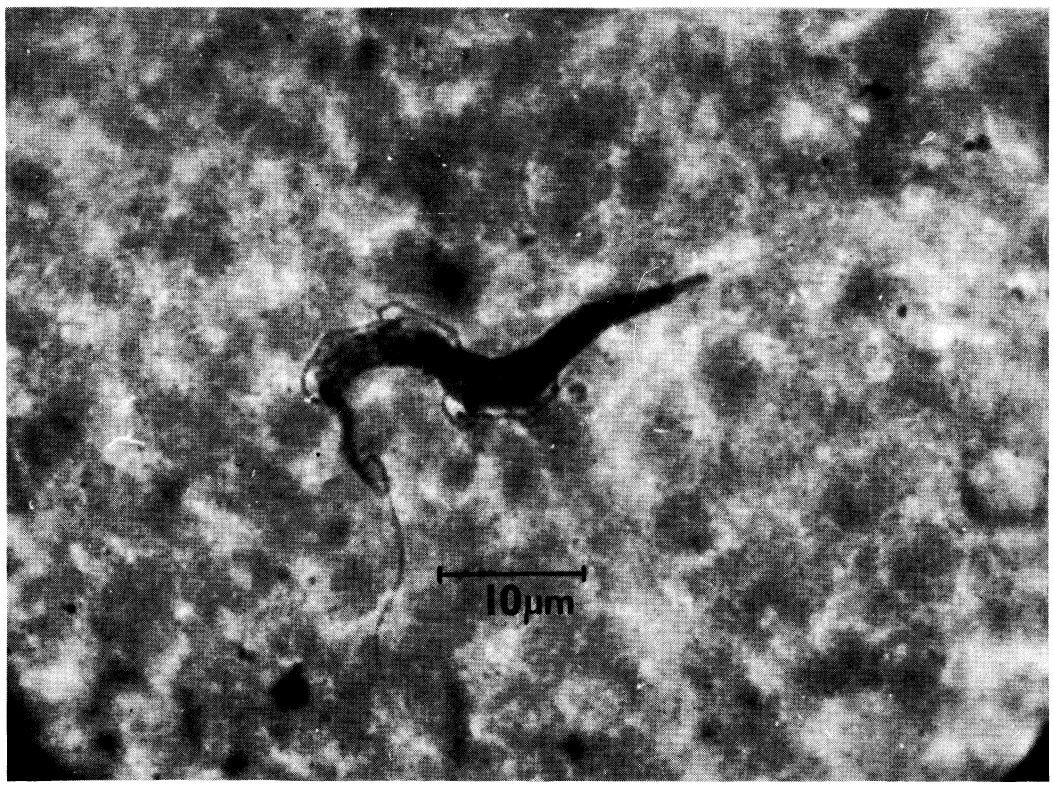


7

(*Facing p.* 56)



10



11

FIGURE 10. Photograph of a typical broad *theileri*-like trypanosome in a thin blood smear from duiker no. 226. Immediately posterior to the band-like nucleus there is a white vacuolated area. Adjacent to the latter is the round dark-staining kinetoplast. Longitudinal striations or 'myonemes' are visible both in the anterior and posterior parts of the body.

FIGURE 11. *Theileri*-like trypanosome in a thick blood smear from the duiker (*Sylvicapra grimmia*). Parasite from antelope no. 322 which showed a low level of parasitaemia. This organism corresponds to Theiler's 'ordinary form', the kinetoplast being situated at some distance from the nucleus, which in this parasite shows as a very dark staining oval area near the middle of the body.

parasite is occupied by the pale pinkish-staining nucleus which is surrounded by a thin halo of pale-blue cytoplasm. In mature microgametocytes (*b*), however, which measure from 5 to 6 μm in diameter, the cytoplasm is difficult to see and the parasite consists almost entirely of nucleus which stains a delicate salmon-pink colour and usually contains one or two small round, darker-staining areas of chromatin. Much less chromatin is present in the immature macrogametocytes (i and ii), about half the parasite being occupied by the pale-blue cytoplasm. The nucleus is usually roundish or oval in shape (ii and iii) and the chromatin stains with about the same intensity as in the microgametocytes. Sometimes, however, the nucleus of immature forms is irregular in shape and the parasite stretches across the infected cell in the form of a band (i). The mature female gametocyte is roughly the same size as a mature microgametocyte and also completely fills the host cell (iii).

In all the recognizable stages of gametocytes, and in asexual forms of the signet-ring and later stages, the pigment is present in the form of small chunky blocks (5*a* to 12, *a*, *b*, ii and iv) or finer rectangular blocks more like those seen in *P.* (*V.*) *cephalophi* (figure 7, plate 28) and Keymer (1966) but not so thick or so long and often with pointed ends (i and iii). Pigment granules are most often clumped together, as seen in the majority of the illustrated parasites, but they sometimes are scattered (12, i and iii). The appearance of pigment in splenic impression smears was discussed by Keymer (1966).

Especially in late stages of the infection, and as early as the 6th day in the splenectomized duiker, two parasites were frequently seen in one erythrocyte (3*a* and iv). This phenomenon, however, was not observed in the naturally infected wild duikers.

Histology and pathology

Approximately 300 serial sections each of the liver from two of the infected wild duikers and 200 from the splenectomized animal were examined histologically for exoerythrocytic stages, but without success. Pigment was present in the Küpffer cells, as was noted in the *P.* (*V.*) *cephalophi* infection, and large quantities were also seen in impression smears of the bone marrow.

No anaemia was apparent in the relatively lightly infected wild duikers, but in both the captive antelopes it became rather severe in the late stages of the infection. Both animals lost weight, although most of the time continuing to retain their appetites. The mucous membranes of the mouth, and the conjunctival membranes became pale in colour. The pelage of both also became harsh and staring, losing its lustre. The latter clinical signs, however, may have been partly caused by a respiratory infection which developed in both duikers before they were infected with malaria and which gradually became more severe as the anaemia became worse. On microscopical examination of the blood, the anaemia first became apparent on the 22nd day of the infection in the splenectomized animal and on the 16th day in the other duiker. Anisocytosis was marked in the late stages of the infection whilst a slight degree of punctate basophilia, polychromasia and poikilocytosis was also present.

Vector investigations

The vector of this parasite is unknown and the speculation regarding the vector of *P.* (*V.*) *cephalophi* discussed by Keymer (1966) applies equally well to this *Plasmodium*.

Over 300 *Anopheles* mosquitoes collected from pens containing captive duikers were dissected for sporozoite infections and for the presence of oocysts, but unfortunately with negative results.

A total of 53 wild-caught *Anopheles* mosquitoes were induced to feed on the infected splenectomized duiker. Feeding experiments were commenced on the 4th day of parasitaemia, but owing to the necessity of having to stop work in Malawi they had to be discontinued on the 11th day. Unfortunately this was 4 days before exflagellation was first definitely observed. This factor, together with the low numbers of microgametocytes present in the blood, undoubtedly diminished the chances of success. All the mosquitoes were kept for periods varying from 3 to 15 days before being fixed in Carnoy's fluid, and a number were later examined histologically for oocysts, but without success.

Discussion

This species of *Plasmodium* differs from *P.* (*V.*) *cephalophi* in several respects (table 1) but the mature microgametocytes are similar to the immature microgametocytes of *P.* (*V.*) *cephalophi* although the mature forms of the latter are larger and stain more darkly.

TABLE 1. MAIN DISTINGUISHING FEATURES BETWEEN *PLASMODIUM* (*VINCKEIA*) *BRUCEI* AND *P.* (*V.*) *CEPHALOPHI*

	malaria parasite	
	P. (*V.*) *brucei*	*P.* (*V.*) *cephalophi*
type of parasitaemia in natural infections	cryptic infections (e.g. 43 parasites per thin blood film). Marked prevalence of asexual forms over gametocytes	parasites exceedingly rare (*e.g.* maximum of 2 per thin blood film). Marked prevalence of sexual forms over schizonts
mature schizonts	reach 8 μm diam. Contain 16 merozoites	reach 8 μm diam. Contain 24–34 merozoites
macrogametocytes	5–6 μm diam. 'Volutin' granules absent	6–8 μm diam. 'Volutin' or chromatin granules usually present
microgametocytes	5–6 μm diam.	6–8 μm diam.
appearance of pigment granules in mature parasites	slender rods often with pointed ends and smaller chunky fragments. Up to 6 and occasionally more in number. Pigment sometimes scattered	relatively long and thick rectangular rods measuring up to 3 μm in length. Usually single. Seldom more than 3 and not scattered
appearance of infected erythrocyte	slight increase in size and loss of density especially when infected by mature gametocyte	usually an increase in size with all mature stages. Density seldom affected
length of schizogonic cycle	*ca.* 48 h	*ca.* 72 h

Macrogametocytes of *P.* (*V.*) *brucei* might be confused with those of *P.* (*V.*) *cephalophi* when the characteristic deeply staining volutin granules usually seen in the latter are absent. The mature female gametocytes, however, are generally larger than those of *P.* (*V.*) *brucei*.

According to Garnham (1964) this *Plasmodium* should be placed in the subgenus *Vinckeia* together with all other *Plasmodium* spp. occurring in mammals below the simian level. Nevertheless, in spite of the fact that it is different in many respects from *P.* (*V.*) *cephalophi*, which as pointed out by Keymer (1966) does not entirely fulfil the characteristics defined for the subgenus, there is also some doubt if this parasite can be placed in the subgenus.

(*b*) *Family Trypanosomatidae*

(i) *Trypanosoma* (*Megatrypanum*) spp. Hoare, 1964, of the duiker and other ruminants.

History and introduction

Trypanosoma theileri was originally discovered early in this century in the blood of an ox by Theiler in South Africa. It was named after him by Bruce (1902) and described by Laveran (1902). Since that time the same or a similar trypanosome has been found on many occasions in the blood of domestic and wild ruminants and almost in every part of the world where these animals are found. Stockman (1910) was the first person to report the parasite in cattle in Great Britain and the first report in a wild ruminant was by Dutton, Todd & Tobey (1906), who found it in the blood of a bushbuck (*T.* (*T.*) *scriptus*) shot in Kasongo in the Congo. Most of the subsequent reports of the trypanosome in wild ruminants have also been in African antelopes. Descriptions of the parasite have usually been based on the measurements and other morphological features of a small number of trypanosomes, because the level of parasitaemia is normally very low.

During the first few years after the original discovery of *T.* (*Megatrypanum*) *theileri* a number of trypanosomes which differed only slightly from the original description of the parasite were considered to be different species. It was soon realized, however, that they were in fact *T.* (*M.*) *theileri*; Wenyon (1926) listing no fewer than 13 synonyms. There has hitherto been insufficient evidence to determine whether or not similar trypanosomes of antelopes, known under the names of *T. ingens* Bruce *et al.* 1909, *T. tragelaphi* Kinghorn *et al.* 1913 and *T. cephalophi* Bruce *et al.* 1915, are all *T.* (*M.*) *theileri* or really represent one or more different species. Nevertheless, several authorities, including Wenyon (1926) and Hoare (1949), have expressed doubts regarding their specific status, especially that of *T. cephalophi* and *T. tragelaphi*, whilst Curasson (1943) believed that *T. ingens* was a 'variation' of *T. theileri*. Reichenow (1940) took the opposite view and not only doubted if the parasites of game and cattle were the same species, but also considered the possibility of different species of *theileri*-like trypanosomes occurring in cattle. In fact in the early part of the century, the 'game species' referred to above were actually recorded in cattle by Bruce *et al.* (1909*b*, 1911*a*), Hamerton (1911), Mackie (1911) and Robinson (1929). When, therefore, during the present survey it was discovered that the incidence of *theileri*-like trypanosomes in duikers was very high, in an effort to clarify this long standing problem, large numbers of blood smears were made from the animals in order to try to obtain as many parasites as possible and enable the morphology to be studied in more detail than previously. The morphology of these duiker parasites has been compared with published descriptions of the trypanosomes in other species, including cattle. For further comparison morphological studies have been made on similar parasites in a blood smear from an Indian ox (*Bos taurus*) in the collection of the London School of Hygiene and Tropical

Medicine, and in a smear from a Nigerian ox (*B. taurus*) kindly lent by Mr E. A. Wells at the Royal (Dick) Veterinary College of Edinburgh University.

In addition to mensural morphological studies of the parasite in different species of bovines, attempts have also been made to culture the organisms, to transmit it by blood inoculation into various splenectomized animals of different species and to find the vector. The results of this work are described and discussed with reference to similar experiments carried out by others.

Distribution and incidence

Geographical distribution. As previously stated, trypanosomes of the *T.* (*M.*) *theileri* type are cosmopolitan and have been reported from cattle in Africa, Europe, Asia, America and Australia. Wild ruminants have been found infected in Europe, Asia and S. America, but mainly in Africa, where these trypanosomes are widespread.

Species incidence. It will be seen from table 2 that *theileri*-like trypanosomes have been recorded in at least 23 different species of ruminants excluding domestic cattle (*B. taurus*), 14 of which are African antelopes. The parasites have also been found in the European bison (*Bison bonasus*), the domesticated Indian buffalo (*Bubalis bubalis*) and domestic zebu (*Bos indicus*), all of which belong to the family Bovidae. *Theileri*-like trypanosomes have, however, been recorded in the lesser Malay chevrotain (*Tragulus javanicus*) also known as the Asian 'mouse deer', which belongs to the family Tragulidae, and in three species of South American deer (*Mazama* spp.) of the family Cervidae. In the latter animals the trypanosome was given the name *T. mazamarum* by Mazza, Romaña & Fiora (1932).

When considering the species of African antelopes in which the parasites have been found, it will be noted that the common duiker and the bushbuck appear to be the most commonly infected (table 2). This may well be a fairly accurate indication of the species incidence in African antelopes, because in the last 60 years thousands of game animals have been slaughtered in Africa and their blood examined for the presence of trypanosomes.

During the present investigation a total of 148 wild ruminants representing 15 different species were examined, but the duiker and puku (*Kobus vardoni*) were the only species in which *theileri*-like trypanosomes were found.

Mention must be also made here of *T.* (*M.*) *melophagium* Flu, 1908, and *T.* (*M.*) *theodori* Hoare, 1931. Both closely resemble *T.* (*M.*) *theileri* morphologically, the former being common in sheep throughout the world whilst the latter is found in goats in Palestine. Hoare (1923) described the life-cycle of *T.* (*M.*) *melophagium* in detail and it is generally accepted to be a distinct species, primarily because it is believed to have a different life-cycle from *T.* (*M.*) *theileri*; it also has different cultural requirements (Herbert 1964). *T.* (*M.*) *theodori*, however, according to Levine (1961) may be a synonym of *T.* (*M.*) *melophagium.*

Goats were common in the Chitala area of Malawi and adjacent to the uninhabited district where duikers were infected with *theileri*-like trypanosomes. Nine goats belonging to Africans were examined for the parasites but without success and four splenectomized goats kept at the base at Chitala apparently remained free from infection in spite of being exposed to possible vectors. The possibility of goats being infected in the area cannot be excluded on this evidence, however, because *T.* (*M.*) *theodori* has not yet been seen in blood smears owing to the cryptic nature of the infections with this organism.

TABLE 2. DATA ON *THEILERI*-LIKE TRYPANOSOMES OF RUMINANTS (EXCLUDING DOMESTIC CATTLE *BOS TAURUS*)

hosts, and percentage of animals infected	'species' of trypanosome	total length (*L*) range (μm)	no. of trypanosomes	authors
family: Bovidae				
subfamily: Bovinae				
bushbuck (*Tragelaphus* (*T.*) *scriptus*)	*T. ingens*	—	—	Bruce *et al.* (1909*b*)
	T. ingens	—	—	Bruce *et al.* (1911*b*)
	T. theileri	43·5–87·0	—	Dutton *et al.* (1906)
	T. ingens	—	—	Fraser & Duke (1912)
	T. ingens	—	—	Old (1915)
	T. tragelaphi	*ca.* 59·0 and 67·0	2	Schwetz (1931)
	T. theileri	—	—	Thomas & Neitz (1933)
situtunga (*T.* (*T.*) *spekei*)	*T. ingens*	—	—	Duke (1912)
	T. ingens?	—	—	Kinghorn & Yorke (1912)
	T. tragelaphi	52·5–72·5	5	Kinghorn *et al.* (1913)
greater kudu (*T.* (*S.*) *strepsiceros*), 7·6% of 13	*T. theileri*	—	—	Dias (1960)
eland (*Taurotragus* (*T.*) *oryx*)	*T. ingens*	—	—	Old (1915)
Asiatic or Indian buffalo (*Bubalus bubalis*)	*T. theileri*	—	—	Pease (1909)
	T. theileri	34·0–59·5	20	Haughwont & Youngberg (1920)
domestic zebu (*Bos indicus*)	*T. theileri*	—	—	Yakimoff *et al.* (1933)
	T. tragelaphi	—	—	Thomson (1931)
European bison (*Bison bonasus*)	*T. wrublewskii* (Syn. *T. theileri*)	—	—	Wrublewsky (1908)
subfamily: Cephalophinae				
bay duiker (*Cephalophus* (*C.*) *dorsalis*)	*T. ingens*	—	—	Rodhain (1916)
grey duiker (*Sylvicapra grimmia*)	*T. ingens*	74·0–90·0	—	Rodhain *et al.* (1913*a*, *b*)
2 infected	*T. theileri*	46·0–70·0	—	Rodhain *et al.* (1913*a*, *b*)
14·0% of 7	*T. ingens*	—	—	Bruce *et al.* (1914)
grey duiker (*Sylvicapra grimmia*)				
2 infected	*T. cephalophi*	40·0–50·0	4	Bruce *et al.* (1915)
14·6% of 89	*T. ingens*	—	—	Dias (1960)
6·7% of 89	*T. theileri*	—	—	Dias (1960)
2·2% of 89	*T. tragelaphi*	—	—	Dias (1960)
23·5% of 89	*theileri*-like trypanosomes (total)	—	—	Dias (1960)
88·4% of 26	*theileri*-like	see table 6	—	Keymer, present survey, habitat II*a*, Malawi
45·0% of 20	*theileri*-like	see table 6	—	Keymer, present survey, habitat I*c*, Zambia
28·5% of 49	*theileri*-like	see table 6	—	Keymer, present survey, habitat III*a*, Rhodesia

TABLE 2 (*cont.*)

hosts, and percentage of animals infected	'species' of trypanosomes	total length (*L*) range (μm)	no. of trypanosomes	authors
subfamily: Hippotraginae				
waterbuck (*Kobus (K.) ellipsiprymnus*)				
16·6% of 6	*T. ingens*	—	—	Bruce *et al.* (1915)
	T. theileri	—	—	Thomas & Neitz (1933)
Buffon's kob (*Kobus kob*)	*T. tragelaphi*	—	—	Curasson (1943) quoting Morriss (1940)
Uganda kob (*Adenota kob*), 6·6% of 15	*T. tragelaphi*	—	—	Brocklesby & Vidler (1965)
puku (*Kobus (A.) vardoni*)	*theileri*-like	74·0–80·5	4	Rodhain *et al.* (1913*a*, *b*)
	T. ingens	—	—	Neveu-Lemaire (1943)
20% of 5	*theileri*-like	52·1, 58·0	2	Keymer, present survey, habitat I*a*, Zambia
reedbuck (*Redunca arundinum*)	*T. ingens*	—	—	Bruce *et al.* (1909*b*)
5% of 9	*T. ingens*	—	—	Bruce *et al.* (1914)
	T. tragelaphi	—	—	Kleine & Fischer (1912) quoted by Wenyon (1926)
	T. theileri	—	—	Neveu-Lemaire (1943)
(*Redunca* sp.)	*T. tragelaphi*	—	—	Curasson (1943) quoting Morriss (1940)
roan (*Hippotragus equinus*)	*T. theileri*	—	—	Neveu-Lemaire (1943)
subfamily: Antilopinae				
oribi (*Ourebia ourebi*) 4% of 26	*T. ingens*	—	—	Bruce *et al.* (1914)
steenbok (*Rhaphicerus campestris*) 33·3% of 3	*T. theileri*	—	—	Schwetz (1931)
suni (*Nesotragus moschatus*)	*T. ingens*	—	—	Dias (1960)
2·5% of 394				
1·7% of 394	*T. theileri*	—	—	Dias (1960)
0·25% of 394	*T. cephalophi*	—	—	Dias (1960)
4·5% of 394	*theileri*-like (Total)	—	—	Dias (1960)
subfamily: Caprinae	*T. melophagium*	25·0–60·5	—	Hoare (1923)
domestic sheep and goats (see text)	*T. melophagium*	40·75–53·75	—	Turner & Murnane (1930*a*)
no records in wild hosts	*T. theodori*	No measurements available	—	Hoare (1931)
family: Tragulidae				
lesser Malay chevrotain (*Tragulus javanicus*), 100% of 3	*T. ingens*	70·0–130·0	8	Dodd (1912)
family: Cervidae				
brocket deer (*Mazama rufa toba*)	*T. mazamarum*	body 36·0–39·0, flagellum 8·0–12·0	—	Mazza *et al.* (1932)
M. nemerivagus	*T. mazamarum*	body 50·0–55·0, flagellum 8·0–15·0	—	Mazza *et al.* (1932)
M. simplicicornis 50% of 2	*T. mazamarum*	58·0–75·0	—	Deane (1961)

Sheep and goats, it should be noted, both belong to the family Bovidae, although in a different subfamily (i.e. Caprinae) from any of the previously mentioned species. According to some authorities (e.g. Simpson 1945; Morris 1965), there are five subfamilies in the family Bovidae, and as can be seen from table 2, species representative of each have been found infected with *theileri*-like trypanosomes. Ellerman *et al.* (1953), however, were not convinced that this classification is a natural one and do not recognize subfamilies in the family Bovidae.

Seasonal incidence. It is thought that there is a higher incidence of infection in the rainy seasons than in the dry seasons (Curasson 1925), and the results of the present survey seem to confirm this opinion. Twenty-six duikers were shot in Malawi during the wet season and at least 23 were infected (88·4 %), compared with 49 obtained in Rhodesia during the dry season, when the parasites were only found in 14 animals (28·5 %). Similarly, of 13 duikers shot in habitat I*c* in Zambia during the dry season only three were definitely positive (23 %), whilst of the seven obtained in the wet season the trypanosomes were found in as many as six (85·7 %).

Isolation of trypanosomes by cultural methods

Introduction. Herbert (1964, 1965) has made detailed studies of the cultural requirements of *T.* (*M.*) *theileri* and the subject will not be reviewed here or discussed in detail. It has been known for many years that both this flagellate and *T.* (*M.*) *melophagium* will readily grow in NNN (blood and agar–agar) medium. This medium was originally devised by Novy & McNeal and modified by Nicolle (1907). Because of its unsuitability for subculture, however, many workers have preferred to use the medium after enrichment with nutrient broth, as originally used by Nöller (1917). With this medium, growth of some strains of *T.* (*M.*) *theileri* only occurs at 28 °C (82·4 °F), according to Herbert (1964). In Nigeria, Wells *et al.* (1965) found that growth of trypanosomes from cattle on blood–agar–glucose was better at 'verandah temperature' than at 27 °C (80·6 °F), the latter temperature presumably being the higher of the two. Sergent & Sergent (1911), using 'ordinary bouillon' in tubes, readily cultured the cattle trypanosomes at 20 °C (68 °F) and stated that the parasites were apparently in the trypanosome form. However, according to Ristic & Trager (1958), at lower temperatures the organisms occur mainly in the crithidial forms, similar to those seen in the invertebrate host, and only in the trypanosome forms when incubated at 37° C (98·6 °F).

Although *T.* (*M.*) *theileri* in the blood of cattle has been successfully cultured on numerous occasions, few attempts appear to have been made to culture the antelope trypanosomes. Bruce *et al.* (1915) attempted to culture *T.* (*M.*) *cephalophi* from duiker blood. They did not state the type of medium used, but of five tubes inoculated only one showed growth. After 11 days, numerous 'actively motile herpetomonad flagellates' were present but died off after a week. Subcultures from this tube showed very scanty or no growth and 'soon died'. The importance of using a large inoculum when culturing *theileri*-like trypanosomes is well known and undoubtedly the sooner the culture is made after the blood has been obtained the more likely the chances of success. Unlike the pathogenic trypanosomes, however, *T.* (*M.*) *theileri* will remain alive in fresh blood for as long as 2 days or even more (Theiler 1903). Bruce *et al.* (1915) unfortunately provided no information concerning the amount or freshness of the inoculum which they used.

Methods. In the present investigations, primary isolations were incubated without any form of artificial heat, as these facilities were not available and heparinized blood was used. Herbert (1964) always used defibrinated or citrated blood, however, and stated that he had no experience of the effect of other anticoagulants on the trypanosomes. No antibiotic was incorporated in the primary cultures, although owing to the difficulties of avoiding contamination under field conditions it was found necessary to incorporate penicillin for subcultures. If this work was repeated, however, antibiotics would be added to all culture media to reduce contamination.

About 1 ml. of blood was inoculated into each of five tubes containing a slope of NNN medium. This was done within 5 min of withdrawing the blood from the heart of the shot duiker and usually as soon as the animal was dead. Sterile disposable syringes were used and the end of the needle was heated in the flame of a spirit lamp before inoculation of the medium. The cotton wool used for plugging the entrance of the tube was not replaced until the aperture had been flamed in the usual way. An attempt was always made to carry out the culturing on the leeward side of the Landrover to reduce the risk of contamination by wind-blown particles. Less frequently inoculation of the culture medium was made under cover at the base camp. In spite of these precautions, however, practically all the cultures became contaminated. This was probably because the heat produced by the flame of the spirit lamp was insufficient to sterilize the needle of the syringe. It was also impractical under field conditions to sterilize the exterior surface of the heart before inserting the needle, although the risk of contamination by this means was reduced to the minimum by always taking blood from the heart as soon as the organ was exposed and without handling or otherwise contaminating its surface.

Results. Attempts were made to culture trypanosomes from the blood of five duikers and a splenectomized calf, all of which showed the parasite in blood smears. Three of the five cultures from duikers became positive and growth was first confirmed on the 9th, 15th and 22nd days, although it may well have occurred more quickly than the two late recorded growths suggest, because owing to the risks of contamination cultures were not examined frequently. The culture of the calf's blood unfortunately became contaminated and no parasites were found.

All the cultures were dispatched to London at the first opportunity, where proper examinations, subculturing and attempts to reduce contamination were carried out.

In no case was a good growth obtained, and the organism in subcultures did not thrive satisfactorily in spite of the removal of contamination, and trial incubation of different tubes at 28 °C and room temperature. Attempts to adapt the trypanosome to growth on Wenyon–Noguchi medium failed and all growth on the modified NNN medium gradually faded away. The culture which first grew on the 9th day survived the longest, last being seen alive after 5 months. Subculturing was carried out at approximately weekly intervals and the cultures thrived most satisfactorily at 28 °C.

Detailed morphological studies of cultural forms were not made although whip-like forms having a long handle-like body with a bulbous end containing the nucleus were observed. A long flagellum appeared to arise at about the same level as the nucleus; no trypanosome forms were observed.

Conclusions. The ability of wild strains of *theileri*-like trypanosomes to grow on artificial

media of the type used in these experiments is known to vary considerably (Herbert 1964). This worker drew attention to the fact that although some media will successfully support the growth of some strains, other strains will completely fail to grow. It cannot be concluded therefore from the above experiments and the experience of Bruce *et al.* (1915) that the duiker trypanosomes grow less readily than *T.* (*M.*) *theileri* of cattle. It has been proved, however, that growth can be obtained when using heparinized blood and also even in the presence of contamination. Undoubtedly, given more time and better facilities than were available during the present investigations, much useful work could be done regarding the cultural requirements of *theileri*-like trypanosomes of game.

Transmission experiments

Introduction. Numerous attempts have been made by many workers to transmit *theileri*-like trypanosomes by blood inoculation to other animals. On a number of occasions success has been reported by inoculating blood from infected cattle into other cattle free from the infection (e.g. Laveran 1902; Theiler 1903; Schein 1907; Behn 1910; Peter 1910; Curasson 1925; Carpano 1932). According to Curasson (1943) *T. theileri* of cattle can be transmitted to goats and sheep, but the parasites die within 8 days; some workers, however, have failed to transmit the trypanosomes to these animals, e.g. Theiler (1903). All attempts to infect other species with the *theileri*-like trypanosomes of cattle appear to have failed; and similarly attempts to infect other species of animals, including ruminants, by inoculation of infected blood from game animals, have also been unsuccessful. The experimental animals which have been used include laboratory and wild mice, rats, rabbits, guinea-pigs, dogs, monkeys, horses, goats and sheep.

Methods. Surprisingly enough, nobody appears to have inoculated *theileri*-like trypanosomes into splenectomized animals or to have attempted transmission of the game trypanosomes into cattle. It was decided therefore to see if the duiker trypanosomes could be transmitted by blood inoculation into the following splenectomized species: a calf, goats, immature duikers, rats and mice. Non-splenectomized immature and adult duikers were also used.

It must be mentioned here that although no trypanosomes could be found in the blood of these animals by the examination of smears, prior to the inoculation of infected blood, the possibility of their already being infected cannot be excluded. It was intended to culture the blood of all ruminants before inoculation, but owing to transport and other difficulties in Malawi there was a long delay in receiving culture media from London and it became necessary to commence the transmission experiments before the media were available because of lack of time.

Curasson (1943) pointed out that it is necessary to inject as much as 5 to 10 ml. of infected blood when attempting transmission into an ox, the incubation period then being 4 to 6 days. If smaller doses are injected, the incubation period is longer. In the present experiments (table 3), relatively large doses were also used.

Results. It will be seen from table 3 that splenectomized animals are apparently no more susceptible to the parasite than those which have not had their spleen removed, *theileri*-like trypanosomes being found in the blood of the splenectomized calf and in one entire duiker after inoculation.

Conclusions. The calf first showed parasites in its blood 4½ days after injection with trypanosomes from a wild duiker and 7 days after splenectomy. For the reasons previously given it is not known for certain whether or not this animal was infected before inoculation, but the presence of a large form over 80 μm in length only 4½ days after inoculation suggests that it may have been previously infected. As discussed later, these large forms are believed by some authorities to denote chronicity, whilst the shortest incubation periods previously recorded appear to be 4 to 6 days when a large inoculum is used (Curasson

TABLE 3. *THEILERI*-LIKE TRYPANOSOMES OF THE DUIKER (*SYLVICAPRA GRIMMIA*): TRANSMISSION EXPERIMENTS

ref. no. of wild duiker	experimental animal*	age†	sex	route	volume of inoculum (ml.)	period of time after death of wild duiker (min)‡	no. of days blood examined	result
227	duiker (E)	ad.	♂	I/P	2	60	—	—
322	duiker (S)	im.	♂	I/M	2	immed.	46	neg.
319	duiker (S)	im.	♂	I/M	3	immed.	3	neg.
319	duiker (E)	im.	♂	I/M	3	immed.	51	neg.
314	duiker (E)	ad.	♀	I/M	5	30	26	pos. 16th and 24th days
314	ox (S)	im.	♂	I/M	4	40	47	pos. 4½, 9, 13, 17, 19, 20, 21, 23, 25, 26, 28, 30, 32, 35, 36, 37, 38, 42 and 46 days after injection
313	rat (S)	—	—	I/P	2	25	19	neg.
313	mouse (S)	—	—	I/P	1	25	19	neg.
312	rat (S)	—	—	I/P	1·5	15	21	neg.
312	mouse (S)	—	—	I/P	1	15	21	neg.
311	goat (S)	ad.	♂	I/M	2·5	20	5	neg.
311	rat (S)	—	—	I/P	1·5	20	31	neg.
311	mouse (S)	—	—	I/P	0·75	20	2	neg.
310	goat (S)	ad.	♂	I/M	3	immed.	5	neg.
310	rat (S)	—	—	I/P	2	immed.	36	neg.
310	mouse (S)	—	—	I/P	1·5	immed.	6	neg.
309	goat (S)	ad.	♂	I/M	3	immed.	38	neg.
309	rat (S)	—	—	I/P	1	immed.	37	neg.
309	mouse (S)	—	—	I/P	0·5	immed.	37	neg.

* E, entire; S, splenectomized. † ad., adult; im., immature.
‡ immed., immediately.

1943) and 4 to 5 days (Levine *et al.* 1956). The inoculated blood showed a low level of parasitaemia, and further support for the belief that the calf was not infected by the trypanosomes which were inoculated is provided by the failure to infect two immature splenectomized duikers by blood inoculation with the duiker parasites (see tables 3 and 4). It is reasonable to expect that they would be more susceptible than the calf by virtue of their being the same host as that from which the parasites were originally obtained. Unlike the calf, the two duikers had been kept in a brick building since they were first obtained shortly after birth. The chances of their having acquired an immunity to the infection by previous contact were therefore remote and further reduced by splenectomy. Considering the apparent ease with which cattle can be infected by blood inoculation of

the parasites from other cattle, if it is assumed that the animals were in fact originally free from infection, it is perhaps surprising that these two immature duikers did not eventually show the trypanosomes in their blood. One reason for this may have been because of the low level of parasitaemia in the donors. Unfortunately, heavily infected duiker blood or that showing evidence of dividing trypanosomes was not inoculated into experimental duikers.

The possibility that the two splenectomized duikers, and indeed any of the other apparently non-infected experimental animals, developed a light infection which was not detectable by examination of blood smears cannot be entirely ruled out. This is thought unlikely, however, considering the large numbers of thick blood smears (usually four)

TABLE 4. DATA ON *THEILERI*-LIKE TRYPANOSOMES INJECTED INTO RUMINANTS

reference no. of wild duiker	experimental animal*	intensity of infection in wild duikers	mean total length (*L*) of parasites (μm)	total length (*L*) range of parasites (μm)	'species' of trypanosome based on 'total length' (*L*) character
227	duiker (E)	low	45·0	42·0–51·25	*T. cephalophi*
322	duiker (S)	low	50·8	42·0–59·1	*T. theileri*
319	duiker (S)	low	87·4	75·75–110·5	*T. ingens*
314	ox (S)	low	95·4	74·1–108·2	*T. ingens*
311	goat (S)	low	73·8	62·5–82·5	*T. ingens*
310	goat (S)	low	not measured	—	—
309	goat (S)	low	parasites only seen in fresh blood	—	—

* E, entire; S, splenectomized.

which were examined daily over quite a long period of time (see table 3). Ideally the blood of these animals should have been cultured periodically but cultures were not made from the duikers because they were needed for other experimental work with *Plasmodium* parasites, and in view of the difficulty of obtaining these animals it was undesirable to jeopardize their chances of survival by excessive handling and withdrawal of blood samples.

It can be seen from table 3 that in spite of the apparent failure to infect splenectomized ruminants, one adult non-splenectomized duiker showed parasites in its blood. These appeared on the 16th and 24th days after inoculation with infected blood which was taken from the same wild duiker as that from which blood was taken for inoculation of the calf. For the reasons already given, however, it seems unlikely that this animal was infected by blood inoculation, especially, as unlike the splenectomized duikers but in common with the calf, it had to be housed outside and was continuously exposed to natural infection.

Comparative morphology of theileri*-like trypanosomes of ruminants*

Introductions. Owing to the low level of parasitaemia which is usually present with infections of these parasites, insufficient material has been available until now to enable comparative biometrical studies to be carried out on the morphology of the organisms.

The morphology of *T.* (*Trypanozoon*) *evansi* has been studied by Hoare (1956) using biometrical methods, also that of *T.* (*Nannomonas*) *congolense* and *T.* (*N.*) *dimorphon* (Hoare 1959) and *T.* (*N.*) *simiae* (Hoare 1936*a*). Similar work has been done on *T.* (*Duttonella*)

vivax and *T.* (*D.*) *uniforme* by Hoare & Broom (1938), *T.* (*D.*) *vivax* by Fairbairn (1953), *T.* (*N.*) *congolense* by Godfrey (1960, 1961) and Fairbairn (1962), and *T.* (*Pycnomonas*) *suis* by Stephen (1963). All these parasites, however, show relatively little variation compared with that of *theileri*-like trypanosomes. The nearest approach to the present studies was the work done by Davis (1952) on *lewisi*-like trypanosomes.

The above workers have studied much more material than was available for this investigation and therefore it might be thought that the present attempt to clarify the taxonomic status of the various species of *theileri*-like trypanosomes on morphological grounds is premature. There is perhaps some justification for this opinion. Over 60 years, however, have elapsed since these trypanosomes were discovered and several different species have been created on much less evidence than is available now. If an attempt to clarify the problem is delayed until the amount of material available compares with that used in biometrical studies on other trypanosomes, then probably at least another 60 years will elapse. This would not matter if more was known about the life-history, biochemistry or serology of the group, but at present there is insufficient knowledge available on these and other aspects of the subject to enable an opinion to be expressed about the taxonomic status of the various species in the group. Because there are statistical methods available to enable comparisons to be made between small samples, and also because during the present survey the collection of *theileri*-like trypanosomes which has been made from duikers is probably the largest collected so far, it is considered that in the absence of a more accurate method and within its limitations the present biometrical study is both justified and desirable.

The present studies are based mainly on examination of *theileri*-like trypanosomes from duikers and cattle, including records of other workers where these provide sufficient information to enable comparisons to be made. A total of 110 duikers from various localities was examined for trypanosomes and 49 (44·5 %) were shown to be infected. At least 150 parasites were found in blood smears, but only 126 were suitable for morphological studies and included in the present work. These trypanosomes were compared with over 75 found in the blood of three oxen and with published records of *T.* (*M.*) *theileri* from two other domestic cattle. Less detailed comparisons, owing to insufficient information, were also made with published descriptions of the trypanosomes in other oxen (including one parasite described as *T.* (*M.*) *ingens*) and in an Asian domesticated buffalo. Similar comparisons were made with *T.* (*M.*) *melophagium* in sheep, *T.* (*M.*) *cephalophi* in a duiker, *T.* (*M.*) *ingens* in one bushbuck, two reedbucks (*Redunca arundinum*) and a Malay chevrotain, *T.* (*M.*) *tragelaphi* in a situtunga (*Tragelaphus* (*T.*) *spekei*), *T.* (*M.*) *mazamarum* in three South American deer (*Mazama* spp.) and with *theileri*-like trypanosomes found in a puku in the present survey. Considering the number of times *theileri*-like trypanosomes have been recorded in ruminants it is remarkable how briefly and inadequately the morphology has been described, especially as the records from cattle exceed the large number listed in table 2 from wild ruminants.

General morphology. The general appearance and internal structure of the *theileri*-like trypanosomes has been described on so many occasions in the past (e.g. Wenyon 1926; Curasson 1943; Levine 1961; and others) there seems little point in dealing with this aspect of the morphology in detail here, because the parasites found in the duikers and also

studied in the blood of three oxen did not differ essentially from previous descriptions. One important morphological feature was noticed, however, that does not appear to have been noted previously, namely the considerable difference in appearance which is often seen between parasites from the same animal and in the same blood sample, when examined in both thick and thin smears. Seldom have past workers stated if their descriptions of parasites were based on thick or thin blood smear examination, but as can be seen from figure 8, showing comparisons of width measurement from both types of preparations, this point is extremely important. The width range in trypanosomes from thin smears is

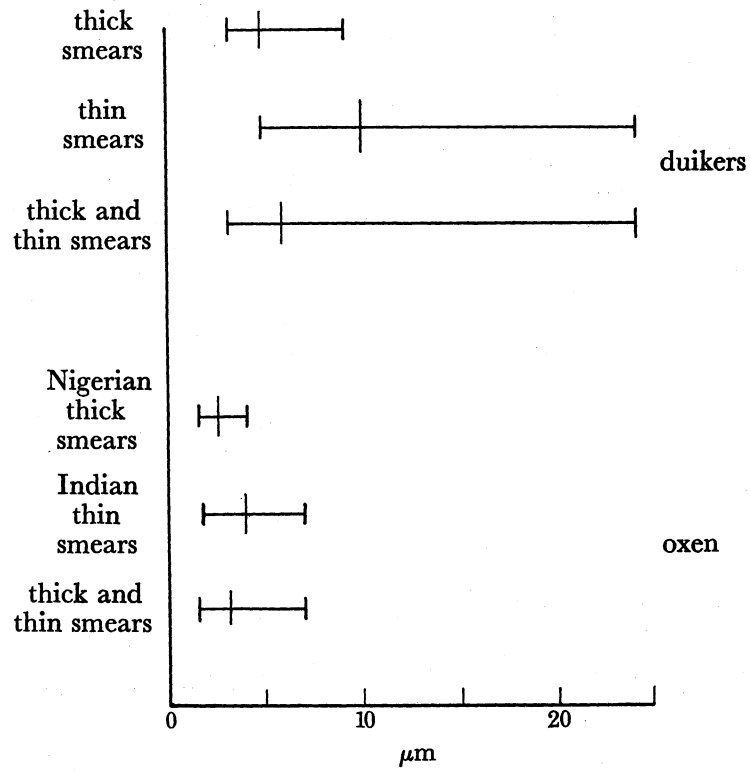


FIGURE 8. Comparison of widths of *theileri*-like trypanosomes in thick and thin blood smears, showing mean values and ranges.

seen to be over 19 μm compared with only 6 μm in thick smears, resulting in a difference of about 5 μm in the mean measurement between both types of preparations. The difference is probably due to the large size of the organism, which results in its being flattened and expanded when thin smears are made (figure 9, and figure 10, plate 29). This flattening also alters the general appearance of the parasite in stained preparations. The parasite in thin smears is more transparent, stains lightly and usually shows numerous basophilic granules arranged mainly in longitudinal parallel rows. In some preparations the granules appear as almost distinct lines and have been given the name of 'myonemes' by several of the early workers, e.g. Bruce *et al.* (1909*b*) and Rodhain *et al.* (1913*a*, *b*). Sometimes these 'structures' seem to run transversely or spirally as pointed out by Dutton *et al.* (1906) and Galliard (1925). Obviously electron-microscope studies need to be made because Vivier & Schrevel (1964), using this method to examine the structure of myonemes in a gregarine of the genus *Selenidium*, found them to be tubular fibrils.

In parasites in thin smears the nucleus is usually in the form of a thin rectangular band stretching from one side of the organism to the other, and the undulating membrane is narrow oralmost non-existent (see figure 9 and figure 10, plate 29). Trypanosomes of this type have not been observed in thick smears, but what undoubtedly represent the same phase of the parasite are the long, almost opaque, very dark staining types in which the body is usually constricted at the level of the nucleus (figure 11, plate 29). The kinetoplast, vacuoles, granules and other internal structures clearly visible in thin smears can often only be distinguished with difficulty, depending upon the intensity of the stain. Obviously these marked differences in appearance make many of the statements regarding morphological features, by previous workers of little value, unless the type of smear in which the parasites were observed is specified.

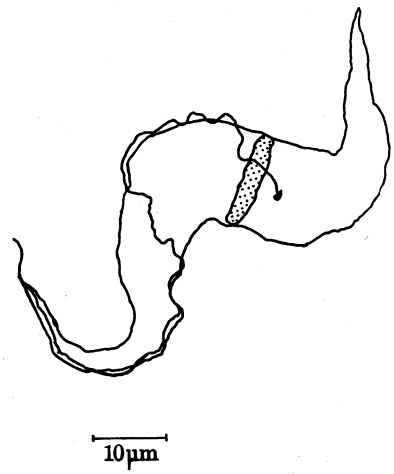


FIGURE 9. *Camera lucida* drawing of typical broad *theileri*-like trypanosome as seen in thin blood smears. This parasite was found in the blood of a duiker (*Sylvicapra grimmia*) no. 237. It showed basophilic granules evenly and thickly distributed throughout the entire cytoplasm, except for the posterior pointed tip. No 'myonemes' could be seen.

Although the width of the organism and its internal morphology differed greatly in thick and thin blood smears there were no statistically significant differences between any other measurements made.

Biometrical studies and methods

Introduction. Biometrical studies on morphological features of trypanosomes are now recognized as an acceptable method of aiding the differentiation of species. This is in spite of the obvious disadvantages such as the heterogenity of samples, variations in fixation and staining techniques, and methods of measuring parasites.

Similar methods to those normally employed by other workers have been used here and the generally accepted method of measuring trypanosomes has already been described.

There are obviously inaccuracies inherent in this method, especially when the results of different workers are compared, and this has been fully appreciated by others. If the method, however, can be used for trypanosomes as small as *T.* (*N.*) *congolense*, measuring as little as 10 μm, then it should be suitable for *theileri*-like trypanosomes which may reach more than ten times this size, because the larger the measurements the less significant are

the small inaccuracies. Giemsa's, or occasionally another Romanowsky stain, has virtually always been used by previous workers and Giemsa's stain was employed in the present study. The fact that this staining technique has been shown to produce variations of 30 % in the mean length of *T.* (*T.*) *brucei* by Walker (1963) is not really as disturbing as it seems, because presumably all parasites would be similarly subjected to this variation and therefore they can be compared.

In addition to the fact that the numbers of samples involved in this study are small and that certain variations occur between parasites in thick and thin blood smears there is one remaining further practical disadvantage in the application of biometrical studies to *theileri*-like trypanosomes, as pointed out by Taliaferro (1923, 1926) and Davis (1952) when studying *lewisi*-like trypanosomes. These workers drew attention to the fact that in *lewisi*-like trypanosomes great variation in size and mean total length of the parasites occur during the course of infection and therefore true mensural comparisons can only be made after organisms have passed through the reproductive and growth stages and have attained a uniform size. The criteria used by Davis (1952) for deciding whether or not the populations were in the adult stage and therefore comparable was calculation of the coefficient of variation (c.v.). With four exceptions (when other material was unavailable) she only compared populations of parasites when the c.v. was 4·5 % or less. *Theileri*-like trypanosomes also show considerable variation; c.v.'s for total length as high as 29·7 % having been calculated for trypanosomes from individual duikers in this investigation, whilst the lowest encountered was 11·2 %. Parasites from the blood of a Nigerian ox showed an even higher c.v., namely 44·1 % (figure 15). Much less is known about the multiplication in the blood of these parasites than of the *lewisi*-like trypanosomes, and it is not known if the *theileri*-like trypanosomes remain a uniform size after multiplication. It seems likely, however, that division and therefore variation, may occur amongst parasites of almost any size and presumably at almost any time during the infection, although probably more often in the early stages. Therefore with these organisms comparisons have to be made irrespective of the size of the c.v. In this work the parasites have been chosen for measurement completely at random and the c.v. has been ignored, it being impossible to arrive at an arbitrary value for c.v. above which comparisons cannot be made.

Statistical data. The three measurements of total length (*L*), distance from the kinetoplast to the nucleus (*KN*) and distance from the posterior end to the nucleus (*PN*) in proportion to the *KN* measurement, i.e. *PN: KN*, of non-dividing parasites are represented graphically by the method devised by Dice & Leraas (1936). Hoare (1956) also used this method when making biometrical studies of *T.* (*T.*) *evansi*.

The statistical constants which have been used are the mean, the standard deviation about the mean and twice the standard error of the mean. When calculating the mean value for any given population every individual trypanosome measurement was used, e.g. when calculating the mean value for measurements of trypanosomes in a geographical population this was not calculated by adding up the mean values for the parasite population in each animal in the locality and dividing by the number of animals, but by the more accurate method of adding up the measurements of individual trypanosomes found in the blood of all the animals in the locality and dividing this figure by the total number

of trypanosomes which were measured, i.e. the number of observations (n) in the locality.

The standard deviation and twice the standard error of the mean were calculated by using the formula given by Bailey (1960) and the coefficient of variation from the formula by Chambers (1943).

In order to use the 'Dice-Leraas diagrams' it is also necessary to note the range of measurements. This is illustrated by drawing a straight line from the point on the graph representing the minimum measurement to another point representing the maximum measurement. The limits of the black rectangles in the diagrams represent the standard deviation, which assuming normal distribution will include the measurements of 68 % of the population. The vertical lines within the white rectangles represent the calculated mean of the observations of the population, whereas these rectangles represent twice the standard error of the mean.

Statistically there is a 95 % chance that the true mean (as opposed to the calculated mean) lies somewhere between the measurements delimited by the outer ends of the white rectangles. The calculated mean values for *L*, *KN*, or *PN:KN* can be considered significantly different from each other statistically, if the white rectangles (twice the standard error of the mean) do not overlap. The wider the gap separating one end of the white rectangle in one population from that in another, the more significant the difference becomes. It has been stated by Dr Trevan (Hoare 1956) that when the white rectangles do not overlap, the probability that they are samples of the same population is not more than 0·05 if the standard deviation of one sample is smaller than that of the other or the samples are of different size. In this study these stipulations were always fulfilled, but as Shaw (1964) has pointed out, the comparison of very small samples below 15, by means of Dice-Leraas diagrams, must be interpreted with care and in the present study the number of samples was often below this figure.

If the end of the black rectangles (standard deviation) do not overlap then at least 84 % of a population is separable from the other, as far as that particular measurement is concerned.

Mensural methods. Measurement of total length of non-dividing parasites is usually regarded as the most reliable morphological quantitative character for biometrical studies, because qualitative characters of trypanosomes are indistinguishable as pointed out by Hoare (1956). In fact it is this measurement which was used by all the previously mentioned protozoologists when carrying out biometrical studies on seven different species of trypanosomes. The measurement has been used in this study primarily because this is the character on which different species of *theileri*-like trypanosomes have been created. It can be seen by referring to figure 12 that when parasites in thick and thin smears are compared, however, total length comparisons are statistically not particularly reliable, the mean length of the trypanosomes in thin smears being 18 μm longer than that in thick smears. There is also a gap of 6 μm between the ends of the white rectangles, the statistical significance of which has already been discussed. The fact that there is no significant difference when parasites from both thick and thin smears are compared with those from thick reduces the importance of the 6 μm gap between the ends of the white rectangles. This is because far less observations were made from thin smears than from thick ones, so that the differences between the parasites in both preparations become reduced.

It appears that in thin preparations the trypanosomes are often stretched lengthwise when the smears are made, as well as in a transverse direction as pointed out earlier. In thin smears it is also quite likely that the flagellum is frequently broken so that a false

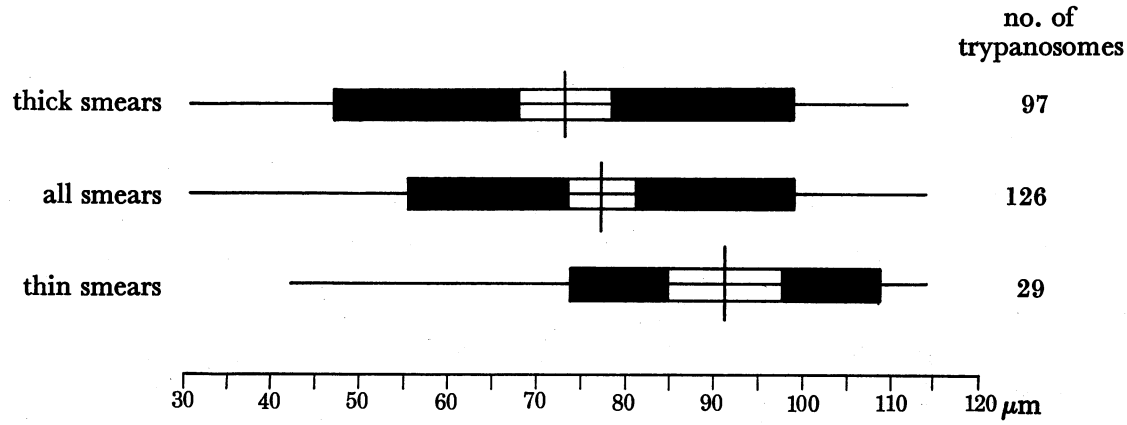


FIGURE 12. Dice-Leraas diagrams. Total length (*L*) measurements of *theileri*-like trypanosomes. Comparisons between parasites from thick and thin blood smears.

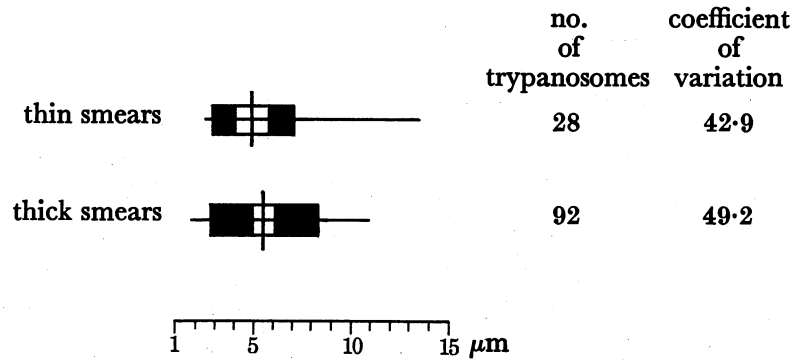


FIGURE 13. Dice-Leraas diagrams. Kinetoplast to nucleus (*KN*) measurements of *theileri*-like trypanosomes. Comparisons between parasites from thick and thin blood smears.

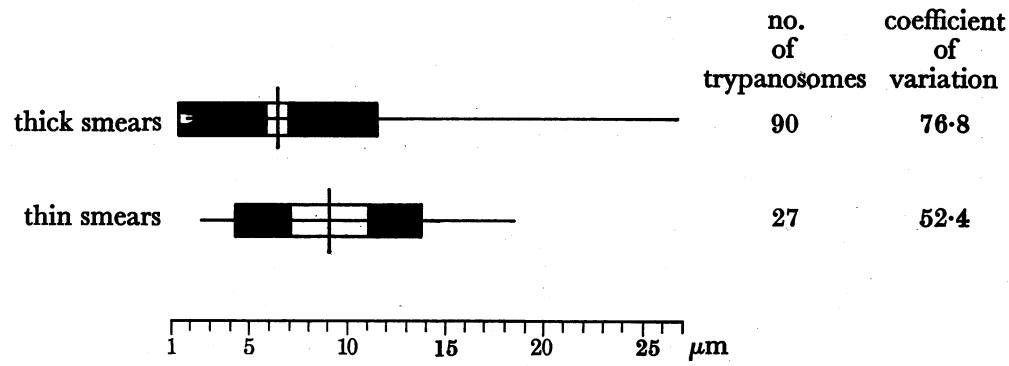


FIGURE 14. Dice-Leraas diagrams. Ratio of (posterior end to nucleus) : (kinetoplast to nucleus) (*PN*:*KN*) measurements of *theileri*-like trypanosomes. Comparisons between parasites from thick and thin blood smears.

measurement is obtained. In addition to comparing total lengths therefore, it was decided to also base comparisons on *KN* and *PN*: *KN* measurements. This ratio, which Keymer (1967) called the kinetoplast index (*KI*), is expressed as a single number obtained by

dividing the distance from the posterior end to the middle of the nucleus by the distance of the kinetoplast from the latter. The ratio measurement was used in preference to *PN* alone, because the *KI* gives at a glance a good idea of the position of the kinetoplast, unlike the *KN* measurement. For example, with a *KN* figure of 5 μm it is not possible, without looking also at the figure for total length, to assess if the kinetoplast is near the nucleus or half way between it and the posterior end. If the trypanosome was 100 μm long obviously the distance of 5 μm is small and the kinetoplast would therefore be close to the nucleus. In a small trypanosome of about 20 μm in length, however, the kinetoplast would be about half-way between the nucleus and posterior end. This is because in all the *theileri*-like trypanosomes the nucleus is generally situated near the middle of the parasite, and therefore the *PN* measurement would be about 10 or rather less than this if the free flagellum was long. If the *KI* figure is 2, the kinetoplast is situated exactly half way between the nucleus and the posterior end. When the *KI* is 3 the kinetoplast is a little nearer the nucleus than the posterior end, whilst high figures such as 12 or more denote it is very close to the nucleus. If the figure is less than 2, then the kinetoplast is nearer to the posterior end than to the nucleus. From figures 12 to 14 it will be seen that *KN* and *PN*: *KN* measurements are the most reliable in this study because unlike *L* measurements the differences are statistically much less marked when duiker trypanosomes from thick and thin smears are compared.

Comparative studies: comparison of trypanosomes from different hosts: measurement of total lengths (*L*). In tables 5 and 6 the total lengths (*L*) of parasites in individual animals of different species are listed, both the mean values and ranges of total length being stated when this information was available.

Owing to the lack of detailed measurements given by previous workers there is usually insufficient information to enable statistical methods to be used and these tables provide the only method of comparing the *theileri*-like trypanosomes known as *T.* (*M.*) *cephalophi*, *T.* (*M.*) *tragelaphi* and *T.* (*M.*) *ingens* with other parasites from cattle recognized as *T.* (*M.*) *theileri* or with *T.* (*M.*) *melophagium* of sheep. It is also the only way, because of the small number of observations and similar lack of detailed measurements, of comparing *theileri*-like trypanosomes of cattle and duikers collected in the present survey, with similar parasites previously recorded in the Asian domesticated buffalo, the puku, bushbuck, situtunga, reedbuck and lesser Malay chevrotain.

Where sufficient evidence was available, the infections have been arbitrarily designated light, moderate, heavy or massive. The number of trypanosomes which were measured are also given, and the species of animal and the source of information are provided.

It can be seen from tables 5 and 6, showing measurements of trypanosomes from mainly cattle and duikers respectively, that although there is a considerable difference between the extreme measurements of 25 and 98 μm for cattle and 40 and 112 μm for duikers, there is nevertheless a fairly regular gradation from the smallest to the largest parasites. It will also be seen by referring to table 2 that the so-called different 'species' of *theileri*-like trypanosomes merge into this gradation of measurements and nowhere do they stand out as being different from the other parasites. Similarly, measurements of parasites from different species of animals, including cattle and duikers, also merge and overlap. There seems little doubt therefore that on purely morphological grounds *T.* (*M.*) *cephalophi*,

TABLE 5. DATA ON TOTAL LENGTH (*L*) MEASUREMENTS OF *THEILERI*-LIKE TRYPANOSOMES AND THE DEGREE OF INFECTION IN RUMINANTS, EXCLUDING THE DUIKER (*SYLVICAPRA GRIMMIA*)

authors	species of host	degree of infection*	number of trypanosomes measured	total length (*L*) range (μm)	total length (*L*) mean (μm)
Keymer (present study)	splenectomized ox (*Bos taurus*)	L	1 (largest parasite)	—	98·0
Dodd (1912)	Malay chevrotain (*Tragulus javanicus*)	L	8 (*T. ingens*)	70·0–130·0	93·2
Saisawa *et al.* (1933)	ox (9. vii. 1927)	H	14	76·1–109·2	87·8
Lady Bruce (unpublished original drawings)	ox	L ?	3	82·5–89·0	85·0
Bruce *et al.* (1909*b*)	? reedbuck (*Redunca arundinum*)	?	5	72·0–122·0	83·0
Saisawa *et al.* (1933)	ox (total infection)	—	31	67·0–109·2	82·9
Keymer (present study)	splenectomized ox 32nd day after injection	L	5	70·5–98·0	82·5
Saisawa *et al.* (1933)	ox (2. viii. 1927)	L	5	77·1–91·5	81·4
Saisawa *et al.* (1933)	ox (18. viii. 1927)	M	7	68·5–89·3	80·3
Lady Bruce (unpublished original drawings)	reedbuck	L ?	1	—	80·0
Keymer (present study)	splenectomized ox, 28th day after injection	L	3	74·75–76·9	75·7
Keymer (present study)	splenectomized ox (total infection)	L	29	47·6–98·0	73·0
Lady Bruce (unpublished original drawing)	reedbuck	L ?	1	—	68·0
Keymer (present study)	splenectomized ox, 37th day after injection	L	2	63·4, 69·3	66·4
Kinghorn *et al.* (1913)	situtunga (*Tragelaphus* (*T.*) *spekei*)	L	5 *T. tragelaphi*	52·5–72·5	63·0
Schwetz (1930)	ox	H ?	2	56·5, 66·0	61·25
Keymer (present study)	splenectomized ox, 30th day after injection	L	2	58·2, 63·6	60·9
Hoare (1923)	sheep	L	1 largest specimen of *T.* (*M.*) *melophagium*	—	60·5
Galliard (1925)	ox	MS	10	—	59·3
Lady Bruce (unpublished original drawings)	bushbuck (*Tragelaphus* (*T.*) *scriptus*)	L ?	2	55·5, 57·0	56·25
Keymer (present study)	puku (*Kobus* (*Adenota*) *vardoni*)	L	2	52·1, 58·0	55·0
Turner & Murnane (1930)	sheep	L	1 (*T. melophagium*)	—	53·75
Schein (1907)	ox	MS	6	45·0–66·2	52·6
Haughwont & Youngberg (1920)	Asian buffalo (*Bubalus bubalis*)	MS	20	34·0–59·5	50·9

* The degree of infection has been arbitrarily defined according to the number of parasites observed in a single thick blood smear. A massive (MS) parasitaemia = 50+, high (H) = 10+, moderate (M) = 6+, low (L) = 5 or less trypanosomes per blood smear.

TABLE 5 (*cont.*)

authors	species of host	degree of infection*	number of trypanosomes measured	total length (*L*) range (μm)	total length (*L*) mean (μm)
Laveran (1902)	ox	L ?	?	—	50·0
Keymer (present study)	Indian ox	MS	25	28·5–68·5	49·6
Hoare (1923)	sheep	(various sources)	5	—	48·3
Keymer (present study)	splenectomized ox	L	(smallest parasite)	—	47·6
Dios & Zuccarini (1924)	ox	L ?	4	34·0–60·0	46·0
Turner & Murnane (1930)	sheep	L	1 (*T. melophagium*)	—	40·75
Keymer (present study)	Nigerian ox	MS	25	18·5–75·45	40·1
Curasson (1925)	ox	L ?	?	25·0–54·0	33·0

* The degree of infection has been arbitrarily defined according to the number of parasites observed in a single thick blood smear. A massive (MS) parasitaemia = 50+, high (H) = 10+, moderate (M) = 6+, low (L) = 5 or less trypanosomes per blood smear.

TABLE 6. DATA ON TOTAL LENGTH (*L*) MEASUREMENTS OF *THEILERI*-LIKE TRYPANOSOMES AND THE DEGREE OF INFECTION IN DUIKERS (*SYLVICAPRA GRIMMIA*)

ref. no.	degree of infection*	no. of trypanosomes measured	total length (*L*) range (μm)	total length (*L*) mean (μm)
192	L	4	98·5–112·0	106·6
313	L	4	90·0–106·5	100·75
236	L	3	87·0–114·0	98·6
35	H	4	89·6–106·4	97·9
212	L	2	96·5	96·5
314	L	18	74·1–108·2	95·4
237	L	3	88·0–98·0	93·0
57	L	1	90·6	90·6
239	M	4	87·0–97·5	90·25
226	L	4	76·0–103·0	88·2
319	L	9	75·75–110·5	87·4
317	L	3	69·25–86·5	77·4
311	L	5	62·5–82·5	73·8
238	L	3	63·0–82·5	73·6
VW 13	L	2	66·6–89·2	72·9
VW 12	L	1	72·7	72·7
318	H	25	30·5–111·8	72·6
D 3	L	2	55·4–85·3	70·3
234	L	2	57·5–82·0	69·7
70	L	4	54·8–73·0	65·5
31	L	5	54·0–97·0	63·2
54	M	6	52·7–73·5	59·3
191	L	1	59·0	59·0
VW 14	L	1	56·0	56·0
322	L	9	42·0–59·1	50·8
227	L	3	42·0–51·25	45·0
Bruce *et al.* (1915)	L	4	40·0–50·0	42·75

* This has been arbitrarily defined as described in table 5. H = high, M = moderate, L = low level of parasitaemia.

T. (*M.*) *tragelaphi* and *T.* (*M.*) *ingens* are invalid species in spite of the opinion expressed by Thomson (1931), who claimed to be able to separate *T.* (*M.*) *tragelaphi*, *T.* (*M.*) *ingens* and *T.* (*M.*) *theileri*. It also appears that at present there is insufficient evidence, as far as the total length characteristic is concerned, to regard *theileri*-like trypanosomes in hosts other than cattle and duikers as different from *T.* (*M.*) *theileri*, or to regard the parasites from cattle and duikers as different species.

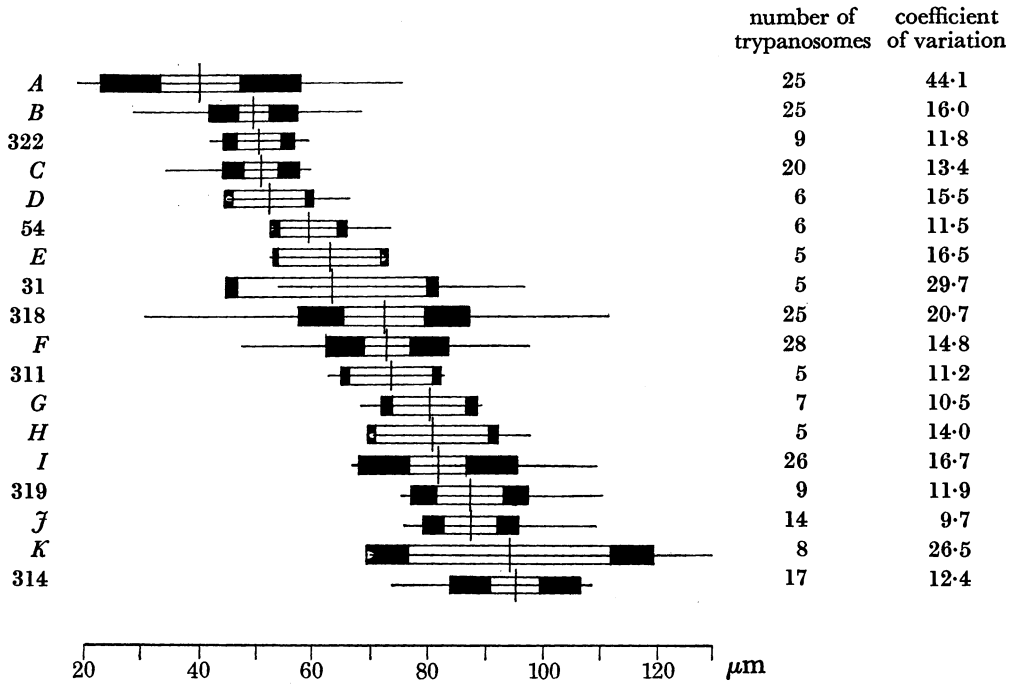


FIGURE 15. Dice-Leraas diagrams. Total length (*L*) measurements of *theileri*-like trypanosomes. Comparisons between parasites from duikers (*Sylvicapra grimmia*), domestic cattle and other ruminants. *A*, Nigerian ox; *B*, Indian ox; *C*, Haughwont & Youngberg (1920), Asian buffalo; *D*, Schein (1907), ox; *E*, Kinghorn *et al.* (1913), situtunga; *F*, splenectomized ox, complete infection; *G*, Saisawa *et al.* (1933), ox, infection on 18 August 1927; *H*, splenectomized ox, 32nd day after injection; *I*, Saisawa *et al.* (1933), ox, complete infection; *J*, Saisawa *et al.* (1933), ox, infection on 9 July 1927; *K*, Dodd (1912), lesser Malay chevrotain; 322, 54, 31, 318, 311, 319, 314, all duikers.

Dice-Leraas diagrams: total length (*L*) *measurements.* It is only possible to use these diagrams for comparative studies when the number of observations is five or more. It will be seen therefore that many of the measurements recorded in tables 5 and 6 can unfortunately not be compared by using statistical methods.

With the diagrams arranged in the ascending order of their means it will be seen (figure 15) that there is a regular gradation between mean values of approximately 40 and 95 μm when trypanosomes from all species are compared. There are also no gaps between the ends of the black or the white rectangles. The populations of parasites from cattle and duikers merge and overlap, so that some populations from either host are inseparable, whilst others are significantly different. Trypanosomes from the Asian buffalo cannot be separated from some of the populations in duikers or in cattle and the same applies to parasites from the situtunga and the Malay chevrotain. Statistical methods therefore

confirm the tentative conclusions drawn by comparing measurements of total lengths given in tables 5 and 6.

Dice-Leraas diagrams: kinetoplast to nucleus (KN) measurements. These diagrams (see figure 16) are arranged similarly to the previous ones. Here it will be seen that the differences are even less evident, 12 of the 15 populations being inseparable, when comparing the white rectangles.

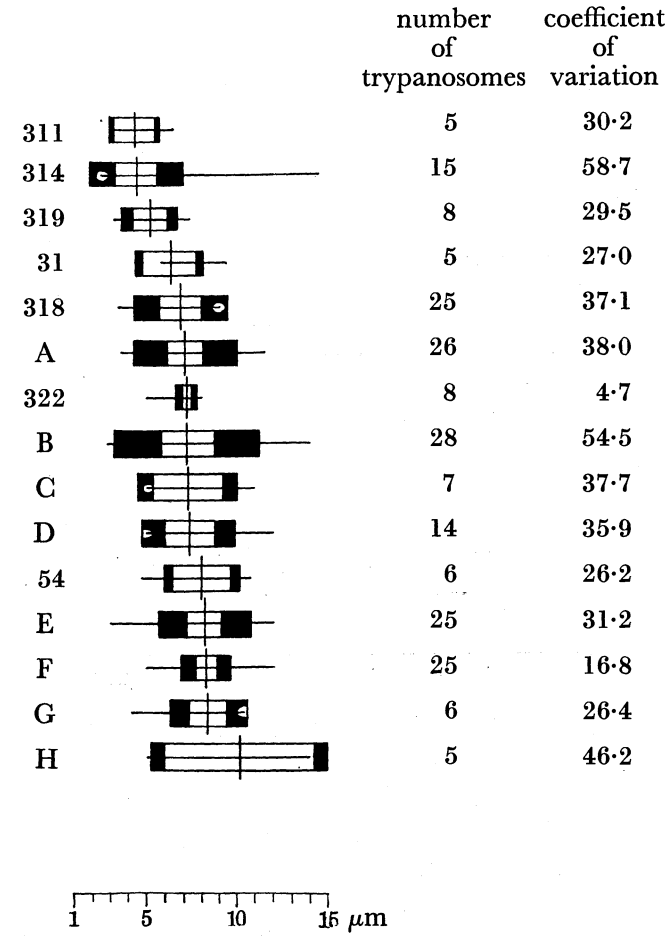


FIGURE 16. Dice-Leraas diagrams. Kinetoplast to nucleus (*KN*) measurements of *theileri*-like trypanosomes. Comparisons between parasites from duikers (*Sylvicapra grimmia*) and domestic cattle. *A*, Saisawa *et al.* (1933), ox, complete infection; *B*, splenectomized ox, complete infection; *C*, Saisawa *et al.* (1933), ox, infection on 18 August 1927; *D*, Saisawa *et al.* (1933), ox, infection on 9 July 1927; *E*, Nigerian ox; *F*, Indian ox; *G*, Schein (1907) ox; *H*, splenectomized ox, 32nd day after injection; 311, 314, 319, 31, 318, 322, 54, all duikers.

Dice-Leraas diagrams: ratio PN:KN measurements. When these measurements are compared (see figure 17) differences between populations are more obvious than when *KN* measurements are used. Nevertheless, as with *L* measurements, a regular gradation is evident from the shortest mean *KI* of approximately 2 in the Nigerian ox to nearly 11·5 in the Malawian duiker no. 314. It will be seen that as with *L* and *KN* measurements no gaps occur between the ends of the white or the black rectangles when these are arranged in ascending order of their means. Trypanosomes from cattle are not completely separable from those of duikers as far as this character is concerned, although some populations in

one species are separable from some populations in the other, e.g. the parasites of duiker no. 314 are statistically completely different from those of the Schein, Nigerian and Indian oxen, but they are also completely different from those of duikers nos. 322 and 31. On the other hand, the black rectangles of 314 overlap with those of the splenectomized ox and the ox of Saisawa, Taise & Kaneko (1933), so that even the most extreme populations of duiker parasites cannot be completely separated from all the oxen parasites as far as this character is concerned.

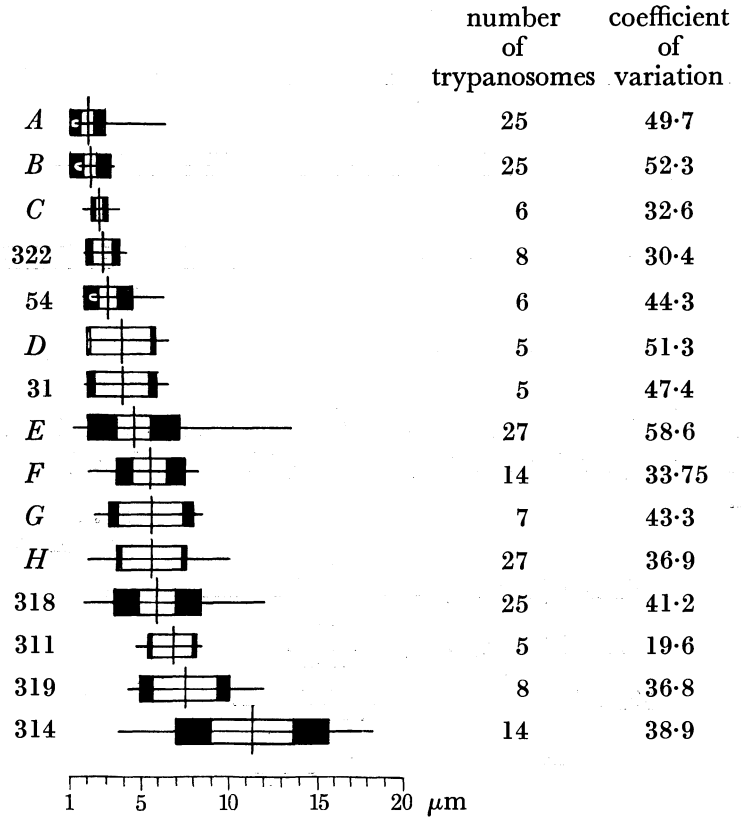


FIGURE 17. Dice-Leraas diagrams. Ratio of (posterior end to nucleus): (kinetoplast to nucleus) (*PN: KN*) measurements of *theileri*-like trypanosomes. Comparisons between parasites from duikers (*Sylvicapra grimmia*) and domestic cattle. *A*, Nigerian ox; *B*, Indian ox; *C*, Schein (1907), ox; *D*, splenectomized ox, 32nd day after injection; *E*, splenectomized ox, complete infection; *F*, Saisawa *et al.* (1933), infection on 9 July 1927; *G*, Saisawa *et al.* (1933), infection on 18 August 1927; *H*, Saisawa *et al.* (1933), complete infection; 322, 54, 31, 318, 311, 319, 314, all duikers.

Comparison of trypanosomes from different localities: Dice-Leraas diagrams (total length (L) measurements). It can be seen from figure 18 that the trypanosomes from the Rumpi district in Malawi can be separated from two of the other three populations (i.e. Rhodesia and Chitala district of Malawi) by comparing the white rectangles. If the black rectangles are compared, however, an overlap occurs and therefore at least 68 % of this Malawian population cannot be separated from the Rhodesian and other Malawian populations.

Dice-Leraas diagrams: kinetoplast to nucleus (KN) measurements. By comparison of standard deviations (black rectangles) in figure 19 the populations of different areas cannot be separated. When the white rectangles are compared, however, the Rhodesian population can be clearly separated from both of the Malawian populations on the basis of this

character, although 68 % of the Rhodesian population cannot be separated because the black rectangles overlap.

Dice-Leraas diagrams: ratio (PN: KN) measurements. Similar conclusions apply as to the *KN* measurements, as can be seen by comparing figures 19 and 20. When the standard

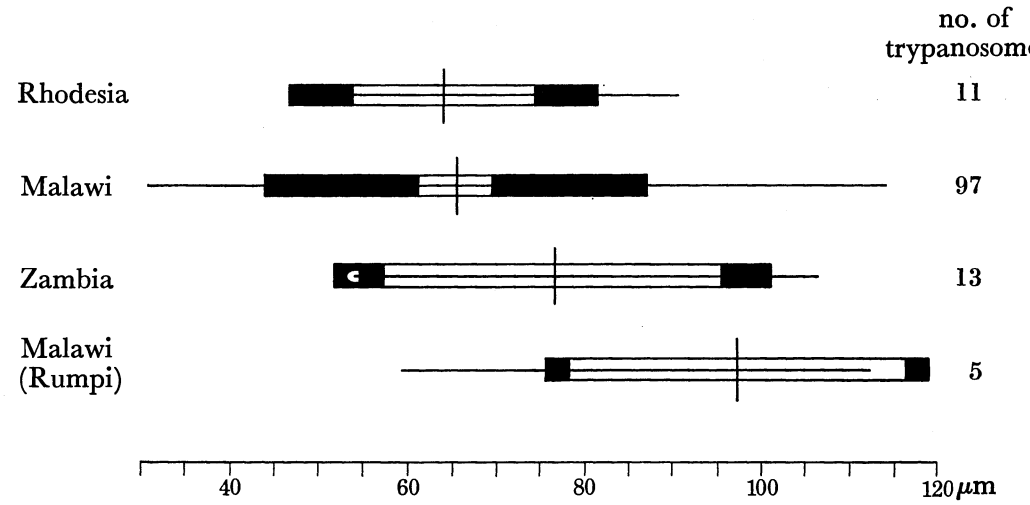


FIGURE 18. Dice-Leraas diagrams. Total length (*L*) measurements of *theileri*-like trypanosomes. Comparisons between parasites infecting duikers (*Sylvicapra grimmia*) in different localities of Central Africa.

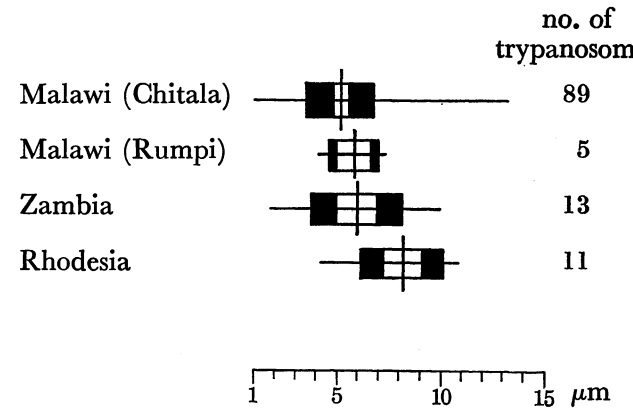


FIGURE 19. Dice-Leraas diagrams. Kinetoplast to nucleus (*KN*) measurements of *theileri*-like trypanosomes. Comparisons between parasites infecting duikers (*Sylvicapra grimmia*) in different localities of Central Africa.

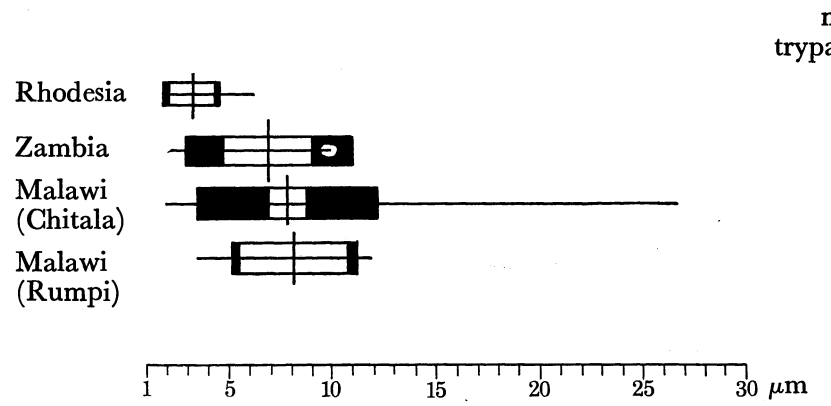


FIGURE 20. Dice-Leraas diagrams. Ratio of (posterior end to nucleus) : (kinetoplast to nucleus) (*PN: KN*) measurements of *theileri*-like trypanosomes. Comparisons between parasites infecting duikers (*Sylvicapra grimmia*) in different localities of Central Africa.

deviations are compared, the Malawi (Rumpi) population can only just be separated from that of Rhodesia.

Conclusions. In spite of the small number of trypanosomes and records of measurements available for biometrical studies, it is obvious that purely on the basis of the three morphological quantitative characters of *L*, *KN* and *PN:KN* there is no clear-cut difference between the trypanosomes in different ruminant hosts or between any of the recognized species in the subgenus *Megatrypanum* (Hoare 1966). Biological characteristics, however, have been demonstrated to justify the existence of *T.* (*M.*) *melophagium* and probably the goat parasite *T.* (*M.*) *theodori* is a 'good species', although Levine (1961) stated that it may be a synonym of *T.* (*M.*) *melophagium.* There is also evidence that *T.* (*M.*) *theileri* of domestic cattle is probably different from these two parasites.

In addition to populations within species, these mensural studies have also shown that there is no significant difference between four different geographical populations of duiker *theileri*-like trypanosomes.

The above conclusions have been formed in a similar way to those of Davis (1952) when making biometrical studies of *lewisi*-like trypanosomes and Hoare (1956) when studying strains of *T.* (*T.*) *evansi.* Both of these workers when comparing measurements of total length found that in spite of wide discrepancies in the mean length among their strains of trypanosomes no real break in variation occurred between one strain and another. Davis stated that, in the group of *lewisi*-like trypanosomes, the inherent variation in size and body proportions of the same organism makes differentiation on a mensural basis alone impossible. This statement applies equally to the *theileri*-like trypanosomes, in which it has been shown by comparison of the coefficient of variations, that the differences in body proportions among populations of these organisms are even greater than those found in populations of *lewisi*-like trypanosomes.

Further support for regarding these parasites as inseparable on morphological grounds is provided by plotting frequency distribution curves (figure 21) of the total length (*L*) measurements of *theileri*-like trypanosomes from a variety of sources. Published measurements from various ruminants and those from duikers and cattle described in this paper are incorporated in curve *a*. The grand total of 314 measurements shows a length distribution ranging from 10 to 130 μm, the variations between the component populations being almost smoothed out. One reason for the slight interruption of the curve could be the fact that some of the measurements quoted by other workers only referred to the shortest and the longest parasites which they found. If measurements of intermediate values had been available, the dip in the curve might have been less marked.

Curve *b* represents 130 *L* values for duiker trypanosomes only, all of which, except for four parasites named *T. cephalophi* by Bruce *et al.* (1915) were obtained during the present survey. In this case the curve is bimodal. In addition to coming from the same host, all the trypanosomes came from fairly similar geographical areas in Central Africa where statistical evidence suggests that the parasite populations are not significantly different. It is unlikely therefore that the bimodal curve represents two different species of parasites and more likely indicates the presence of two types depending upon the stage of infection. The same explanation may also account for the shape of curve *a*. The shape of curves *c* and *d* appear to support this theory because they are both plotted from 25 *L* measurements

of parasites obtained on a single occasion only, in two relatively heavily infected oxen. The parasites are short and virtually all fall within the left hand peaks of curves *a* and *b*. The shape of curve *e* is different from the others and was obtained by plotting 38 *L* measurements of parasites occurring in the blood of the splenectomized calf covering a period of 43 days. The degree of infection in this calf was relatively low, and although the peak of the curve is exactly midway between the lowest level of the bimodal curves

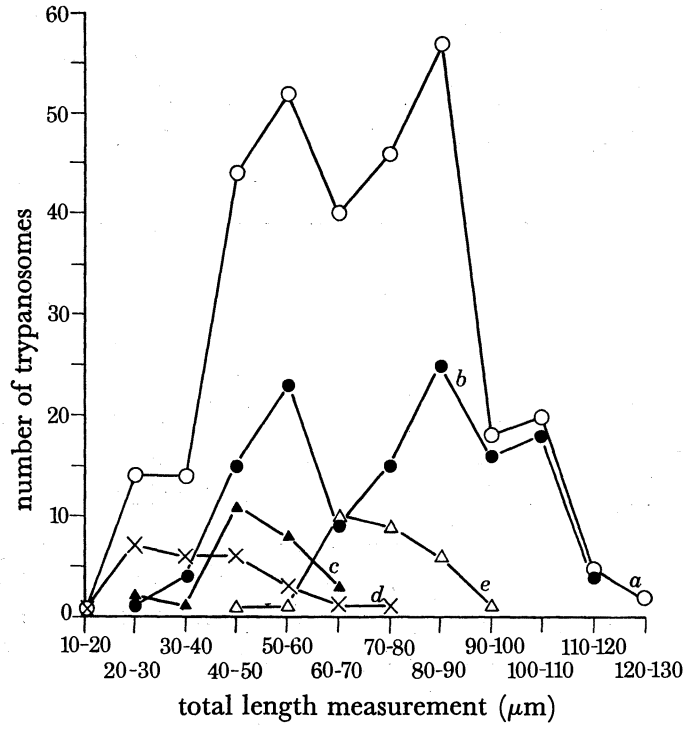


FIGURE 21. Graph showing frequency distribution of total length (*L*) measurements of *theileri*-like trypanosomes from various hosts. (*a*) Parasites from 314 ruminants of various species (data from present survey and published sources). (*b*) 130 *L* measurements; parasites of duiker (*Sylvicapra grimmia*) only. (*c*) 25 *L* measurements; parasites of Indian ox (*Bos taurus*). (*d*) 25 *L* measurements; parasites from Nigerian ox (*B. taurus*). (*e*) 25 *L* measurements; parasites of splenectomized ox (*B. taurus*).

a and *b*, the bulk of the parasites are relatively long and fall within the right hand peaks of *a* and *b*.

Course of blood infection

Introduction. There is very little reliable information concerning the courses of blood infections of *T.* (*M.*) *theileri* in cattle, although it is generally believed that considerable variation in the size of the parasites occurs at different stages of the infection. *T.* (*M.*) *theileri* was stated to multiply in the tissues by Carpano (1932) but Hoare (personal communication) believes that this is incorrect, there being strong evidence that division takes place in the 'crithidial'* stage in the blood. The method of reproduction of *T.* (*M.*)

* Hoare & Wallace (1966) have recommended that this stage should in future be known as the 'epimastigote' stage.

melophagium, *T.* (*M.*) *theodori* and the *theileri*-like trypanosomes of game animals remains unknown.

Multiplication of trypanosomes in the blood. Parasites in the blood which were believed to be dividing were first seen and recorded by Theiler (1903) and have been reported since by other workers including Luhs (1906), Schein (1907), Curasson (1925) and Reichenow (1940).

Theiler (1903), in addition to finding actual dividing forms in the blood, saw trypanosomes with two nuclei but with only one kinetoplast and flagellum. During the present investigations several binucleate trypanosomes were also seen in the blood of an ox from

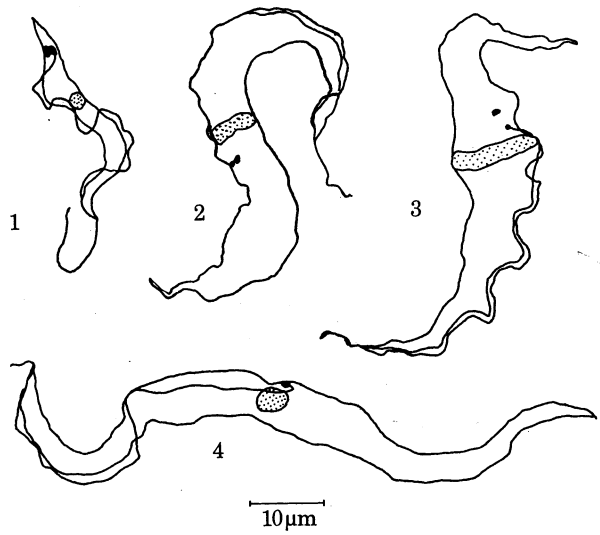


FIGURE 22. *Theileri*-like trypanosomes from the blood of duikers (*Sylvicapra grimmia*) which may be showing evidence of division. (1) Trypanosome in a thick blood smear from no. 318, showing two kinetoplasts lying side by side. (2, 3) Trypanosomes in a thin blood smear from no. 238, both containing two kinetoplasts. (4) Parasite in a thick blood smear from no. 212. Intermediate form between 'crithidial' (epimastigote) and trypanosome stages.

India and relatively fewer in the blood of an ox from Nigeria in which the level of parasitaemia was lower. There is little doubt, however, that these forms are abortive because the final stages of division have not been recorded by any worker. Examples of these types were observed in three duikers out of a total of 49 infected animals; approximately 150 trypanosomes being seen in all these antelopes. In duiker no. 318 a trypanosome was found with two kinetoplasts lying side by side, 5 μm from the posterior end and nearly 8 μm from the nucleus (figure 22 (1)). This is a relatively small parasite measuring just under 50 μm in total length. In another duiker (no. 238) a parasite with two similarly placed kinetoplasts, but only 4 μm from the nucleus and 31·5 from the posterior end, was found (figure 22 (2)). In this animal, however, another trypanosome with two kinetoplasts lying in a vacuole close to the nucleus and approximately 5 μm apart was also seen (figure 22 (3)). The flagellum arises from the kinetoplast which is near (5 μm) the nucleus and situated 27 μm from the posterior end. These forms are much larger than the parasite in duiker no. 318, being 82·5 and 75·5 μm respectively. In duiker no. 212 a form intermediate

between a 'crithidial' (epimastigote) stage and a trypanosome form was seen (figure 22 (4)), in which the kinetoplast is at the same level as the nucleus with the flagellum appearing to arise posterior to it and about level with the posterior border of the nucleus. This parasite is also of the very large type with a total length of 96·5 μm. In duiker no. 318, 14 trypanosomes were present in one thick smear and considerable variation in the length of the parasites also occurred (see table 6 and figure 23). In duikers 212 and 238, however, the infections were light, only four parasites being found in four blood smears (one thick

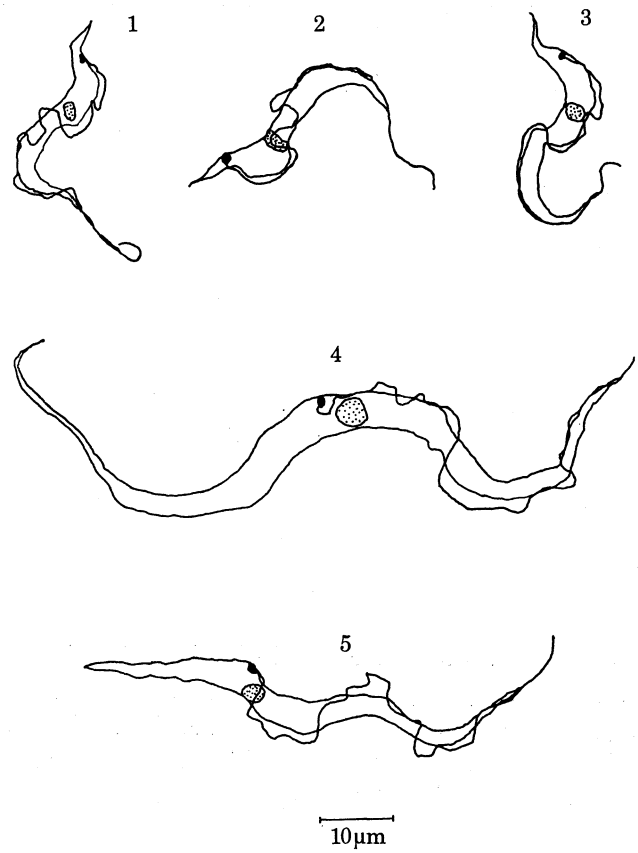
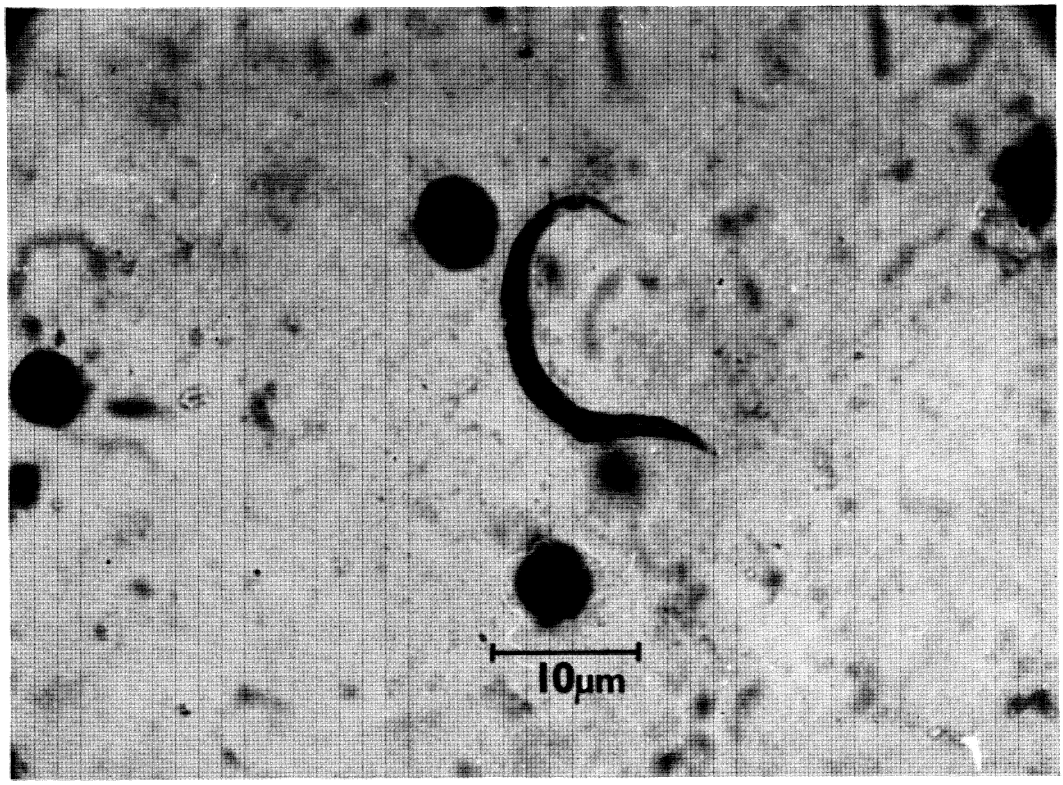


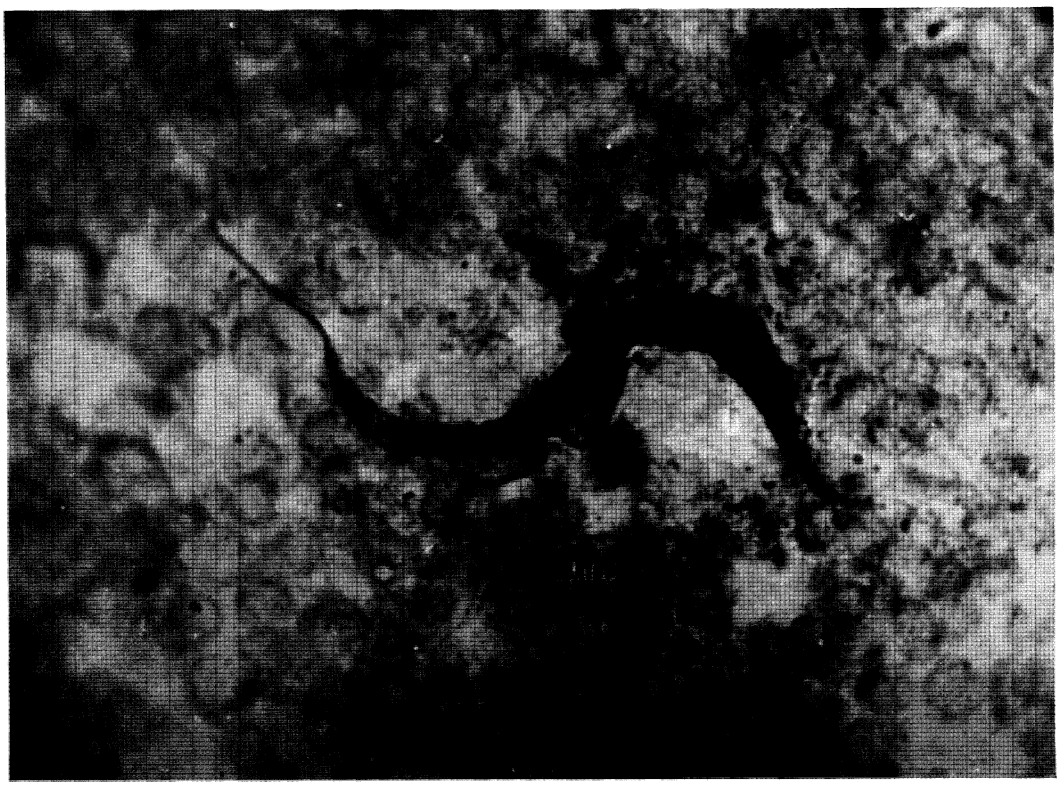
FIGURE 23. *Theileri*-like trypanosomes in thick blood smears from the duiker (*Sylvicapra grimmia*). *Camera lucida* drawings of trypanosomes from a heavily infected antelope (no. 318). 1 to 3 correspond to Theiler's 'ordinary forms', whilst 4 and 5 represent *T. transvaaliense* types.

and three thin) taken from no. 212 and three in three smears (one thick and two thin) taken from no. 238.

Theiler (1903) when studying the original *T.* (*M.*) *theileri* trypanosome found by him in an ox recognized two main types of parasites. A form corresponding to the one described above in duiker no. 318, which he called the 'ordinary form' (see figures 23, 25, 26; and figures 24, 28, plates 30, 31) and another type similar to those found in duiker 212 and 238, described by him as a 'rarer form' (see figures 23, 25, 26; figure 27, plate 30) which at the time it was first seen was given the name of *T. transvaaliense* by Laveran (1903). Theiler stated that the 'ordinary form' varied in length between 20 and 70 μm whilst Laveran gave an approximate average of 30 μm for the *T. transvaaliense* type, stating that



24

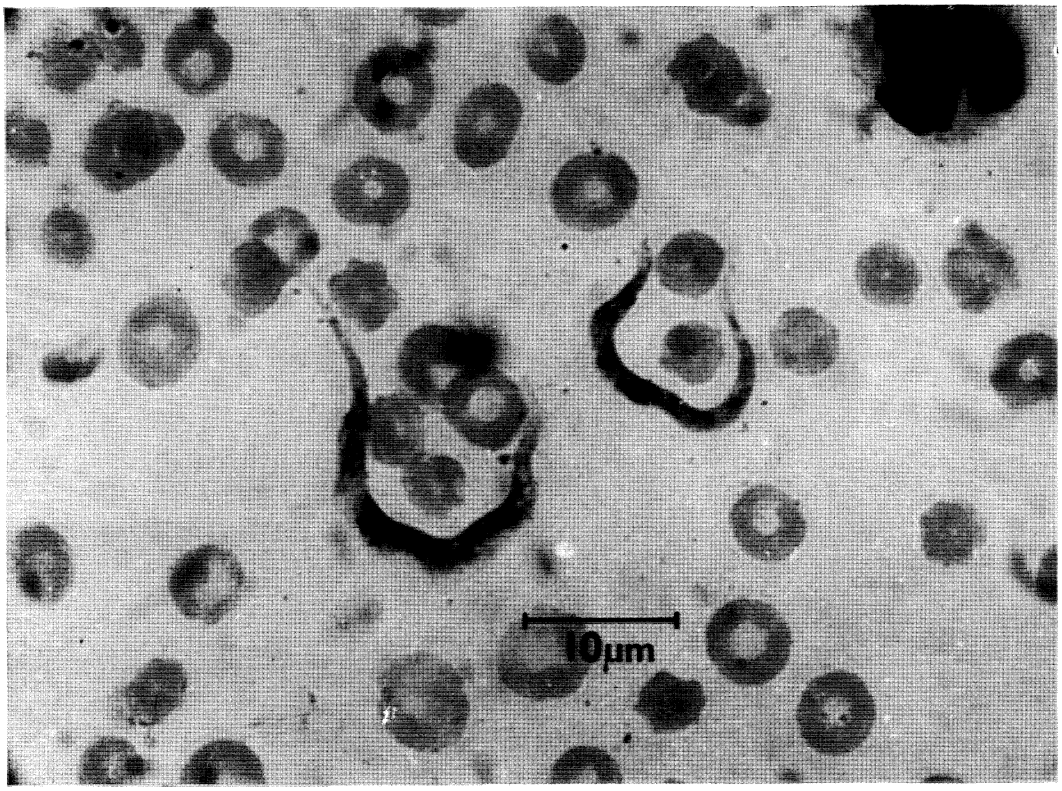


27

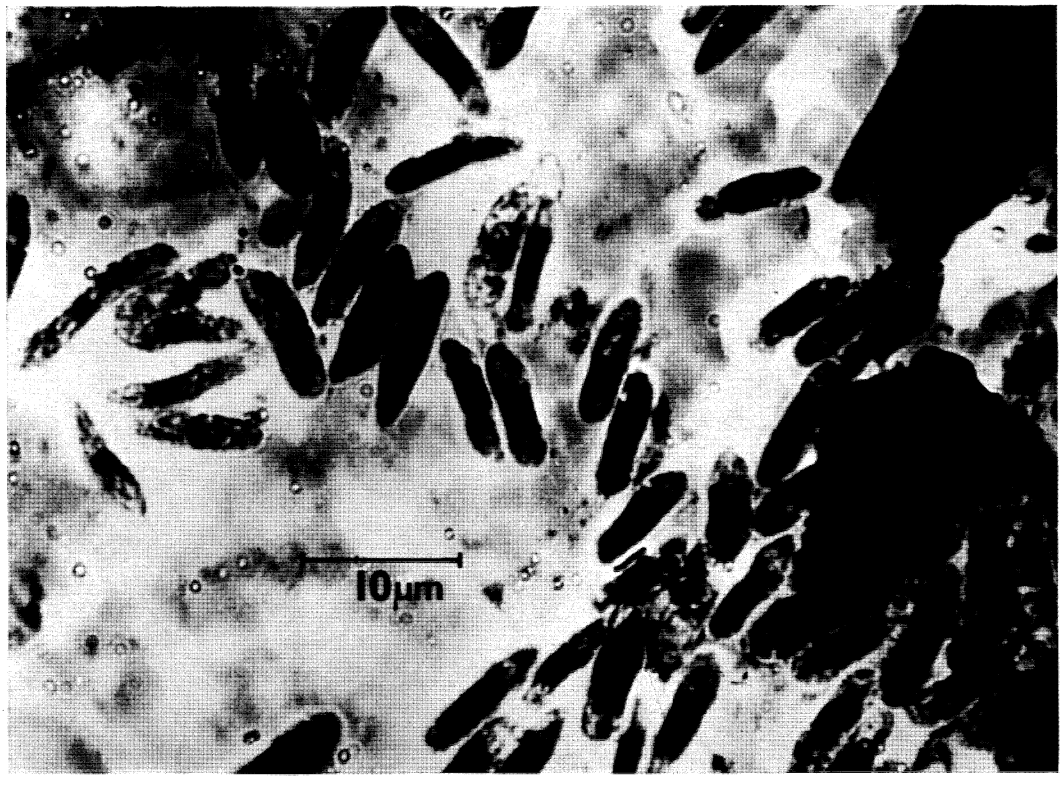
FIGURE 24. *Theileri*-like trypanosome; Theiler's 'ordinary form' with a *KI* value lower than 2, the kinetoplast being nearer the posterior end than the nucleus. Thick blood smear from Nigerian ox (*Bos taurus*).

FIGURE 27. *Theileri*-like trypanosome in a thick blood smear from a splenectomized calf (*Bos taurus*), 36th day after injection. This parasite corresponds to the *T. transvaaliense* type originally described by Laveran (1903). The kinetoplast is near the nucleus, and a considerable distance from the posterior end of the body.

(*Facing p.* 84)



28



32

FIGURE 28. Thin blood smear from Indian ox (*Bos taurus*). *Theileri*-like trypanosomes. The smaller parasite is typical of Theiler's 'ordinary form' with a *KI* value lower than 2. The larger parasite, however, having the kinetoplast rather nearer the nucleus than the posterior end, is intermediate in type between Theiler's 'ordinary form' and the '*T. transvaaliense*' type.

FIGURE 32. Photograph of *Sarcocystis* 'spores' as seen in a thin blood smear from a duiker (*Sylvicapra grimmia*), reference no. 226, from Malawi. The dark staining nucleus is visible in most 'spores', being situated near the centre of the parasite. The cytoplasm is pale staining and granular.

they showed considerable variation in one and the same preparation, the longest being 50 μm. In 'ordinary forms' the kinetoplast is at a considerable distance from the nucleus and often near the posterior end of the body (i.e. it has a *KI* of less than 2), whilst in the other type it is close to the nucleus or occasionally actually lying on it, therefore having a

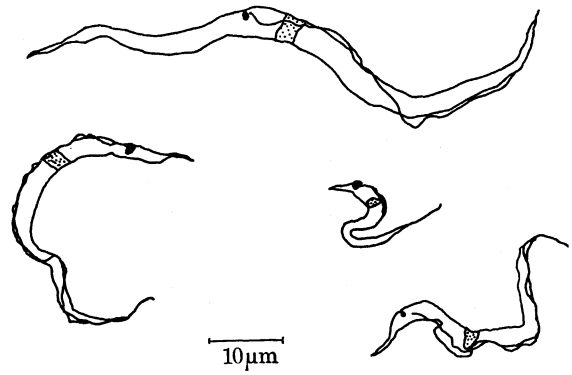


FIGURE 25. *Theileri*-like trypanosomes in a thick blood smear from a Nigerian ox (*Bos taurus*), showing marked variation in the morphology of the parasites. Compare with figures 23 and 26, illustrating trypanosomes in the duiker (*Sylvicapra grimmia*) and a splenectomized ox (*B. taurus*) respectively. In all three animals the so-called 'ordinary forms' and *T. transvaaliense* types are present.

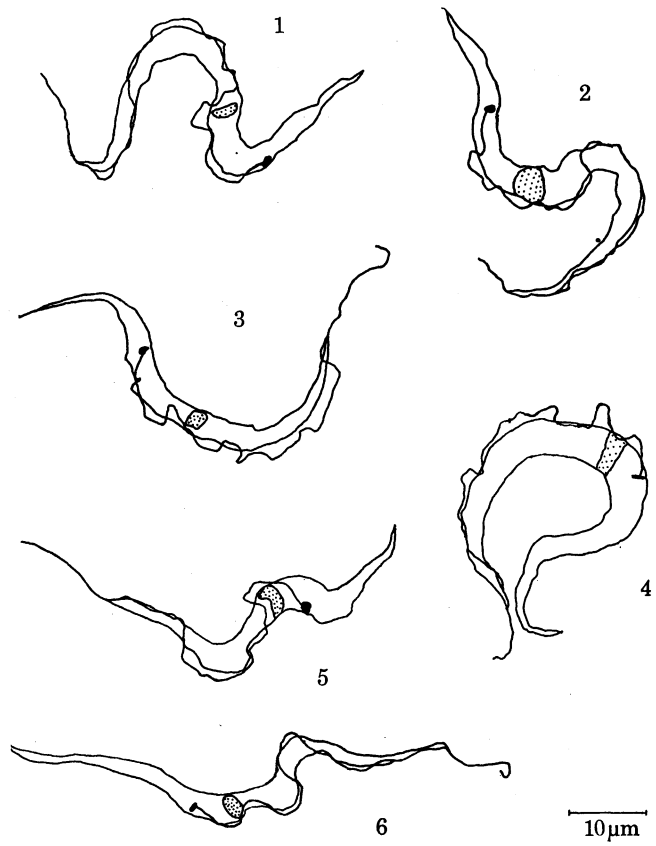


FIGURE 26. *Theileri*-like trypanosomes from thick smears of the blood of a splenectomized calf (*Bos taurus*). *Camera lucida* drawings of trypanosomes, showing Theiler's 'ordinary forms' (1 to 3) and *T. transvaaliense* types (4 to 6).

KI of considerably more than 2. Theiler (1903) found dividing forms of both types but stated that division was less easily demonstrated in the 'rarer forms'. Laveran (1903), whilst discussing Theiler's 'ordinary forms', stated that the longest and largest were generally in the process of division. The situation of the kinetoplast next to the nucleus as seen in the transitional stage described above in duiker no. 212 was stated by Laveran (1903) to be characteristic of *T. transvaaliense*, a name which Wenyon (1926) recognized as a synonym of *T.* (*M.*) *theileri*. In the opinion of Hoare (personal communication) *T. transvaaliense* also comprised 'crithidial' (epimastigote) forms (see coloured plate depicted by Laveran & Mesnil 1912).

In 1906 Luhs described dividing forms in the blood with two or three nuclei and one or two kinetoplasts. He stated that in the young forms the kinetoplast was close to the nucleus, as in the *T. transvaaliense* type, and their total lengths ranged from as little as 19 to 38 μm. He also recognized two main types of trypanosomes, the other corresponding to Theiler's 'ordinary form' (mainly with a *KI* of less than 2), which Luhs called a 'thin form' and which varied in length from 56 to 68 μm. Luhs, however, did not describe division in trypanosomes of Theiler's 'ordinary form'.

The next year Schein (1907) described trypanosomes in an ox showing evidence of multiplication and with two nuclei. He also drew attention to the 'polymorphism' of the parasite, stating that the kinetoplast was variable in position and often near the nucleus as in the *T. transvaaliense* type (i.e. *KI* greater than 2).

Curasson (1925) recorded multiplication by binary fission in the blood of an ox and stated that 'crithidial' (epimastigote) forms were 'not rare'. He did not describe two types of trypanosomes and stated that the kinetopiast was often situated mid-way between the nucleus and the posterior end, i.e. they had a *KI* of 2 and corresponded to Theiler's 'ordinary forms'.

It was 15 years later that Reichenow (1940) attempted to clarify the significance of the various dividing forms in the blood. Eight years before the appearance of Reichenow's paper, Carpano (1932) had stated that division occurred mainly in certain internal organs and Carmichael (1939) also produced what he considered to be evidence of multiplication in the organs. Reichenow (1940), however, disagreed and stated that all division occurred in the blood.

Reichenow described two main types: long thin trypanosomes reaching a little over 60 μm characterized by having a long free flagellum, and smaller stumpy reproducing forms that immediately after division have a total length of only 20 to 28 μm. He believed that only the long thin forms developed further and changed into the enormous broad forms with much shorter flagella which he considered to be characteristic of chronic infections.

Reichenow stated that both forms are produced by unequal binary fission, one of each being produced during the division.

Conclusions. In spite of the small number of observations, it seems likely that multiplication in the blood of the *theileri*-like trypanosomes is probably similar in all ruminants and takes place in the 'crithidial' (epimastigote) stage.

Morphological changes during the course of infection

Total length (L) in relation to level of parasitaemia. Although Behn (1910) did not state that he found evidence of multiplication in *T. theileri*, he did, like most of the other workers already mentioned (including also Dios & Zuccarini 1924) recognize two main types: a small thin form measuring as little as 22 μm and a large form reaching a length of 70 μm. In all his parasites the kinetoplast was at a distance from the nucleus, being nearer the posterior end of the body in the small forms and half-way between the end and the nucleus in the large form, i.e. the *KI* value was 2 or less. The long forms described by Dios & Zuccarini (1924) measured 50 to 60 μm and the smaller forms 30 to 40 μm. According to Behn and others, large forms appear towards the end of the infection. Richardson & Kendall (1963) seemed to be of the same opinion and Reichenow (1940) stated that the long thin and large broad forms denote chronicity. This implies that large forms are seen mainly when the parasitaemia is low. From tables 5 to 8, however, showing mean lengths, length ranges, *KI* values in short and long parasites, and the degree of infection in different hosts, it will be seen that this is not always the case.

If very long trypanosomes are regarded as those measuring 60 μm or more and *T.* (*M.*) *melophagium* is ignored as being a different species, then in tables 5 and 6, 16·2 % of the infections consisting of trypanosomes exceeding a mean length of 60 μm reached a moderate or higher level of parasitaemia as arbitrarily defined in the tables. When 'very long' is defined as trypanosomes exceeding 55 μm in total length, the percentage is 18·6. Although based on more evidence than that of previous workers this is still only a very approximate method of determining the significance of large forms in the blood because the actual level of parasitaemia in the various animals listed in the table is either unknown or only roughly estimated and what is meant by a 'very long' trypanosome has not been clearly defined by the various authorities. Reichenow (1940) described it as a parasite reaching a little over 60 μm and Behn (1910) up to 70 μm, these being the longest trypanosomes they saw. When the 'very long' thin forms, believed to be characteristic of chronic infections are regarded as 70 μm or more in length, however, their significance becomes more doubtful, because from the table it has been concluded that although 25 % of the infections containing mean lengths under 70 μm were moderate to heavy, as much as 17·2 % of infections showing a mean length of over 70 μm had a relatively high level of parasitaemia. If, on the other hand, infections in species other than cattle are excluded, then there is more evidence to support the opinion that large forms occur in the blood when the parasitaemia is low or the infection is chronic. It can be seen by reference to table 5 that only one ox had an apparently heavy infection of trypanosomes exceeding a mean of 60 μm in length, i.e. that recorded by Saisawa *et al.* (1933). The possibility of two different species of parasites being present, when the two relatively distinct forms are seen in the blood at the same time, can almost certainly be disregarded as first shown by Theiler (1903), because when he inoculated the 'ordinary form' into an ox the *T. transvaaliense* forms were produced.

Position of kinetoplast in relation to the level of parasitaemia. A more accurate idea of morphological changes according to the level of parasitaemia can be obtained by studying the *KI* values and presenting these in the form of contingency tables (tables 7, 8). The results show (table 7) that when the level of parasitaemia is low or moderate (i.e. all duikers and

a splenectomized ox) in at least 75 % of the parasites the *KI* value is more than 2. There is also a slight tendency with higher levels of parasitaemia for the opposite to occur, the kinetoplast tending to be nearer the posterior end than the nucleus, i.e. the *KI* value is less than 2.

TABLE 7. POSITION OF KINETOPLAST (I.E. RATIO *PN: KN* MEASUREMENT OR *KI*) IN RELATION TO THE LEVEL OF PARASITAEMIA IN DUIKERS AND OXEN

hosts and levels of parasitaemia (L, M, H and MS)*	kinetoplast indices (*KI*)								total numbers of parasites measured
	kinetoplast nearer the posterior end than the nucleus (< 2)		kinetoplast midway between posterior end and nucleus (2)		kinetoplast slightly anterior to midway between posterior end and nucleus (2 to 3)		kinetoplast much nearer nucleus than the posterior end (3 >)		
	number	%	number	%	number	%	number	%	
duiker no. 319 (L)	0	—	0	—	0	—	8	100	8
duiker no. 322 (L)	0	—	2	25	3	37·5	3	37·5	8
24 duikers (all L)	3	3·5	1	1·2	7	8·1	75	87·2	86
duiker no. 54 (M)	0	—	0	—	5	83	1	17	6
duiker no. 318 (H)	9	36	2	8	1	4	13	52	25
splenectomized ox (L)	1	3·7	1	3·7	10	37	15	55·6	27
Indian ox (MS)	8	32	2	8	14	56	1	4	25
Nigerian ox (MS)	17	68	5	20	2	8	1	4	25
total L levels	4	3	4	3	20	16	101	78	129
total M levels	0	—	0	—	5	83	1	17	6
total H levels	9	36	2	8	1	4	13	52	25
total MS levels	25	50	7	14	16	32	2	4	50
total	38	18	13	6	42	20	117	56	210
all duikers	12	10	3	3	13	11	89	76	117
all oxen	26	34	8	10	26	34	17	22	77

* The degree of infection has been arbitrarily defined as in tables 5 and 6. MS = massive, H = high, M = moderate and L = low level of parasitaemia.

Position of kinetoplast in relation to host species. In both duikers and oxen the *KI* was usually greater than 2, this being more marked in the former (table 7). As contingency tables 7 and 8 show, contrary to what has been previously believed (Hoare 1938), the explanation for this appears to be more likely due to the low level of parasitaemia in most of the duikers, rather than to any host species difference. Hoare (1938) regarded the position of the kinetoplast as a valuable supplementary character for the differentiation of certain species, such as *T. theileri* and *T. ingens*, stating that in *T. theileri* the kinetoplast is typically circular in outline and subterminal in position (i.e. the *KI* value is 2 or a little less than 2), whereas in *T. ingens* it is subcentral (i.e. the *KI* value is more than 2).

Position of kinetoplast in short and long parasities in relation to total length (L), the level of parasitaemia and the host species (table 8). Separation of the trypanosomes into short and long parasites is based on the lowest point, between the peaks of the dimodal curves labelled *a* and *b*, on the graph showing frequency distribution of total lengths (figure 21). Those trypanosomes measuring less than 65 μm being regarded as short, whilst

the larger parasites are considered to be long. Sixty-six per cent of short and 99 % of long trypanosomes had the kinetoplast nearer the nucleus than the posterior end (i.e. the *KI* value was over 2) when all the duiker parasites were compared. The proportions were similar in the Indian and Nigerian oxen, except that less of the short trypanosomes in these animals had a *KI* of more than 2, the figures being 58·4 % of short and 100 % of long in the former and 8 % of short and 100 % of long in the latter. The parasites of the splenectomized ox differed from these two oxen and the duikers, 100 % of short and 95 % of long

TABLE 8. POSITION OF KINETOPLAST (I.E. RATION *PN:KN* MEASUREMENT) IN SHORT AND LONG PARASITES IN RELATION TO TOTAL LENGTH (*L*)—DUIKERS AND OXEN

host and total length (*L*) measurements	no. of parasites measured	level of parasitaemia	kinetoplast indices (*KI*)			
			< 2·0	2·0	2·1 to 3·0	3·0 >
duikers in present survey						
total	117	mixed	—	—	—	—
< 65 μm	41		12 (29%)	2 (5%)	11 (27 %)	16 (39 %)
> 65 μm	76		0	1 (1%)	2 (3 %)	73 (96 %)
24 duikers: all animals except nos. 54, 318, 319, 322 (see below)						
total	56	L*	—	—	—	—
< 65 μm	14		1 (7%)	0	2 (14%)	11 (79 %)
> 65 μm	42		0	1 (2%)	2 (5%)	39 (93 %)
duiker no. 314						
total	14	L	—	—	—	—
< 65 μm	0		0	0	0	0
> 65 μm	14		0	0	0	14 (100%)
duiker no. 319						
total	8	L	—	—	—	—
< 65 μm	0		0	0	0	0
> 65 μm	8		0	0	0	8 (100 %)
duiker no. 322						
total	8	L	—	—	—	—
< 65 μm	8		2 (25 %)	0	3 (37·5 %)	3 (37·5%)
> 65 μm	0		0	0	0	0
duiker no. 54						
total	6	M*	—	—	—	—
< 65 μm	5		0	0	5 (100%)	0
> 65 μm	1		0	0	0	1 (100%)
duiker no. 318						
total	25	H*	—	—	—	—
< 65 μm	15		9 (60 %)	1 (6·7%)	2 (13·3 %)	3 (20 %)
> 65 μm	10		0	0	0	10 (100 %)
splenectomized ox						
total	27	L	—	—	—	—
< 65 μm	6		0	0	2 (33 %)	4 (67 %)
> 65 μm)	21		1 (5 %)	0	9 (43 %)	11 (52 %)
Indian ox						
total	25	MS*	—	—	—	—
< 65 μm	24		8 (33·3 %)	2 (8·3%)	13 (54·2 %)	1 (4·2 %)
> 65 μm	1		0	0	1 (100 %)	0
Nigerian ox						
total	25	MS	—	—	—	—
< 65 μm	24		17 (71%)	5 (21 %)	2 (8 %)	0
> 65 μm	1		0	0	0	1 (100 %)

* The degree of infection has been arbitrarily defined as in tables 5, 6 and 7. MS = massive, H = high, M = moderate and L = low level of parasitaemia.

having a *KI* of more than 2, i.e. Theiler's *T. transvaaliense* type. They do, however, most closely resemble the duiker trypanosomes, in which there appears to be a greater tendency for short parasites to have the kinetoplast nearer the nucleus than the posterior end, as can be seen from the overall *KI* figures for duikers and for the antelopes nos. 54 and 322 when these are considered separately. This is probably not a host species difference, however, but seems more likely to be related to the level of parasitaemia. When parasitaemia is low as was the case in the splenectomized ox, duikers nos. 314, 319 and 322, and in the collective *KI* figures for 24 duikers, in the vast majority of parasites the *KI* was greater than 2 in both the short and the long, although in duikers nos. 314 and 319 no short trypanosomes were seen. Conversely in the heavily infected duiker no. 318, only 33 % of the short parasites had a *KI* of over 2, compared with 58·4 % for the Indian ox and as little as 8 % for the Nigerian ox, both of which had massive levels of parasitaemia. It will be noted that the trypanosomes of the Indian ox more closely resemble those of the splenectomized than the Nigerian ox, because the majority of short and all of the long parasites have a *KI* greater than 2 in spite of the fact that the level of parasitaemia was designated 'massive'. It is obvious therefore that no definite conclusions can be drawn regarding the position of the kinetoplast in short trypanosomes in relation to the level of parasitaemia or in any of the parasites in relation to the host species. It is clear, however, that in long parasites, irrespective of the host and level of parasitaemia the kinetoplast is virtually always much nearer the nucleus than the posterior end, i.e. the *KI* value is greater than 2.

Course of blood infection in a splenectomized calf. The morphological changes of the parasites which are stated to occur in the blood during the course of infection and which are supposed to lead to the formation of the large forms have apparently never been carefully investigated by regular daily examination of blood smears. It is of some interest therefore, to note the appearance of the trypanosomes and degree of parasitaemia that occurred in the splenectomized calf.

Six thick blood smears were examined daily for 47 days from the time the parasites were first seen and until the calf was killed. As will be seen from figure 29 the infection remained light in spite of splenectomy and trypanosomes were found on only 18 days during this period. At first the parasitaemia was very low, most of the trypanosomes being found after the 17th day. No dividing forms were seen and contrary to the belief of some workers, already discussed, the parasites did not become larger as the infection became more chronic. This may have been because the infection was not followed for a sufficiently long period or because the calf had been infected for some time prior to the blood inoculation and that the majority of parasites had already reached their maximum length. The increase in parasitaemia after the 17th day, however, does suggest that multiplication took place about this time, and as Schein (1907) recorded an incubation period of 13 days and Peter (1910) 9 to 16 days it is possible that this rise in parasitaemia denoted that the calf became infected with the trypanosomes injected from a duiker, in spite of already being infected, splenectomy having lowered its resistance. The evidence against this theory, however, is that the duiker's blood which was inoculated showed a low level of parasitaemia with very large forms averaging 95·4 μm in length and showing no evidence of division (table 4). If, in fact, multiplication was occurring during this rise in the level of parasitaemia then it is interesting to note that the dividing parasites, whether 'crithidia' forms (epimastigotes) or

trypanosomes, were probably of the long thin type, because throughout the observed period of the infection the parasites always exceeded 47 μm in total length. Approximately half of them could probably be regarded as belonging to the *T. transvaaliense* type, whilst the others were similar to Theiler's 'ordinary forms' (see figure 26).

Conclusions. Although, unfortunately, the study of the course of infection in this calf did not reveal dividing parasites in the blood or produce any definite information, the results tend to confirm the doubts expressed in the preceding paragraphs regarding large trypanosomes always denoting chronicity, and multiplying or dividing forms being confined to small parasites. It would be very interesting under vector-free conditions to infect

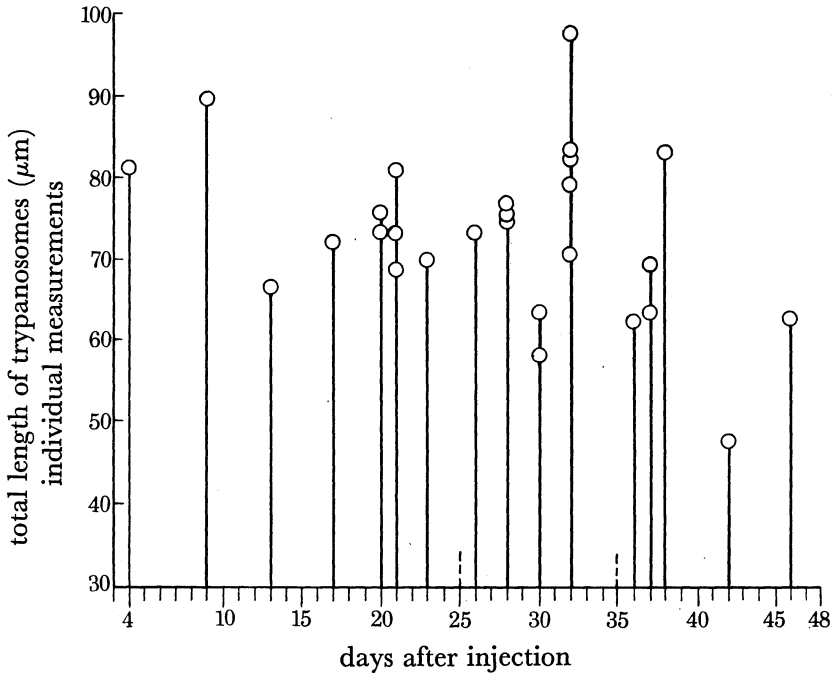


FIGURE 29. Total length (*L*) measurements of *theileri*-like trypanosomes during the course of infection in the blood of a splenectomized calf (*Bos taurus*). The circles show the total length of individual trypanosomes. The dotted lines on the 25th and 35th days after injection represent individual trypanosomes which were unsuitable for measurement.

splenectomized, previously non-infected cattle and duikers, and study the course of the infection daily in the blood over a long period of time. If the trypanosomes were concentrated by centrifugation and by using phytohaemagglutinin in the blood samples that were taken, as described by Yaegar (1960) when studying *T. cruzi*, this problem might well be solved.

It is interesting and undoubtedly of some significance to note that during the course of infection of the splenectomized calf (see figure 29) trypanosomes were found in the blood which on the presently accepted basis of total length measurement, corresponded to four different species, namely *T.* (*M.*) *theileri*, *T.* (*M.*) *cephalophi*, *T.* (*M.*) *tragelaphi* and *T.* (*M.*) *ingens*. On the 4th, 9th, 20th, 21st, 28th, 32nd and 38th days the parasites could be described as *T.* (*M.*) *ingens*, on the 13th, 23rd, 30th, 36th and 46th days as *T.* (*M.*) *tragelaphi* and as *T.* (*M.*) *cephalophi* on the 42nd day after injection with parasites. Furthermore, using the same criterion of species differentiation, the parasites found in the blood of duiker

no. 277 probably corresponded to a mixed infection of *T.* (*M.*) *theileri* and *T.* (*M.*) *cephalophi*; duiker no. 322 *T.* (*M.*) *theileri*, and duikers nos. 31 and 70 *T.* (*M.*) *tragelaphi*, whilst the trypanosomes described by Saisawa *et al.* (1933) in an ox corresponded to *T.* (*M.*) *ingens* (see table 5). The trypanosomes of many of the duikers collected in this survey indeed could not be identified purely on the basis of total length measurements. Even ignoring the statistical evidence, these observations alone make the acceptance of species differentiation of *theileri*-like trypanosomes on morphological grounds of very doubtful value.

Vector investigations

Introduction. It is now generally accepted (Levine 1961) that *T.* (*M.*) *theileri* of cattle is transmitted by dipterous insects of the genera *Tabanus* and *Haematopota* and that, like *T.* (*Herpetosoma*) *lewisi* of the rat and *T.* (*Schizotrypanum*) *cruzi* of man, transmission is contaminative, development of metacyclic trypanosomes taking place in the posterior station. This conclusion is based on the work of Nöller (1916, 1925), Kraneveld (1931) and Wallace (1962). In Africa, however, O'Farrell (1913) and Carpano (1932) provided evidence which led Carpano to believe that the parasite may also be transmitted by the bite of a tick, *Hyalomma aegyptium*, as well as by contaminative means. It was originally believed by Theiler (1903) that transmission was by hippoboscid louse-flies. If the transmission Theiler described did in fact take place, through the agency of these insects, he did not provide sufficient evidence to conclude that hippoboscids were true vectors, because the transmission could well have been purely mechanical.

A hippoboscid, however, has been shown to transmit *T.* (*M.*) *melophagium* of sheep; the sheep ked *Melophagus ovinus* being responsible, as finally demonstrated in England by Hoare (1923), after the discovery of 'crithidial' (epimastigote) stages in the ked by Pfeiffer (1905) and Flu (1908). Similarly, Theodor (1928 and 1929) has shown that the hippoboscid *Lipoptena caprina* transmits *T.* (*M.*) *theodori* of the goat in Palestine and *T. avium* of birds has been shown by Baker (1956) to be transmitted by the hippoboscid *Ornithomyia avicularia*.

In the Chitala area of Malawi, biting flies of the genera *Tabanus* and *Haematopota* were common during the rainy season, whilst hippoboscids of the species *Echestypus paradoxus* were found on 91 % of the wild duikers shot in the area. Similarly a high percentage of the duikers obtained in Zambia were also infected with these parasites, but unfortunately information concerning the incidence of hippoboscids on the Rhodesian duikers is lacking. In view of the high level of infection of *theileri*-like trypanosomes in these duiker populations and the role played by louse-flies in the transmission of the sheep, goat and avian parasites, it was thought desirable to investigate the possibility of hippoboscids transmitting the duiker *theileri*-like trypanosomes in addition to biting flies.

Results. A total of 62 flies of the genus *Haematopota* and 79 of the genus *Tabanus* were examined microscopically for the presence of metacyclic trypanosomes after dissection of the mid- and hind guts, whilst similar examinations of 132 louse-flies (*Echestypus paradoxus*) taken from duikers infected with *theileri*-like trypanosomes were also made.

All of the examinations produced negative results, although on one occasion the presence of metacyclic trypanosomes was suspected in the hind gut of one hippoboscid. The

organisms together with the contents of the gut were mixed with a small quantity of normal saline and immediately injected intramuscularly into an immature duiker which had been splenectomized the previous day. Although its blood was examined almost daily for 2 months, no trypanosomes were found.

On one occasion, when there was insufficient time to carry out microscopical examination of 18 hippoboscids which had been removed from a duiker showing a light infection of *theileri*-like trypanosomes in its blood, the dissected portions of the mid- and hind guts were suspended in normal saline within a few minutes of dissection and immediately injected intramuscularly into an adult splenectomized duiker. The blood of this antelope was subsequently examined daily for a period of 16 days without finding flagellates in the blood.

On a few occasions hippoboscids were not dissected but were fixed in 10% formol saline or Carnoy's fixative. Microscopical examination of sections stained with Giemsa's stain failed, however, to reveal the presence of any metacyclic stages.

Conclusions. Although these investigations failed to prove that hippoboscids are a vector of *theileri*-like trypanosomes of duikers, it is felt that this line of research is worth pursuing further. It is interesting to note that although two adults and one immature splenectomized duiker, in addition to one adult and three immature non-splenectomized duikers, were exposed to possible vectors for several weeks in the Chitala area, where a high percentage of wild duikers harboured *theileri*-like trypanosomes, these parasites were only found in the blood of two of these seven animals. On only three occasions was one trypanosome found in the blood; once in a splenectomized adult and twice in an entire adult. Both animals had been kept in captivity elsewhere for many months and it is quite possible that they had a chronic infection with a very low level of parasitaemia when acquired. This seems particularly likely in view of the failure of the immature splenectomized duiker to become infected, especially as this animal would be expected to be more susceptible to infection than adults and was exposed to possible vectors in the bush for longer periods than any of the other duikers.

It is probable that some of the captive duikers were bitten many times by biting flies, and it is surprising therefore that if these insects are vectors they did not develop levels of parasitaemia comparable with the wild duikers in the same area. The louse-fly *E. paradoxus*, however, is wingless and undoubtedly normally spread by direct contact, which means that the isolated captive animals were less exposed to natural infestations with this possible vector. This was shown by the failure (with one exception) to find louse-flies on the captive animals, in spite of frequent searches being made of the pelage, the immature animals being closely examined almost daily during one period of about 2 weeks. On the occasion when a hippoboscid was found on an immature duiker, it is possible that it was due to contamination from a wild antelope which had been shot and was being autopsied nearby.

Bequaert (1942) made a special study of the Melophaginae of antelopes and other Bovidae and stated that over 150 species had been described at that time. He stated that louse-flies seem to avoid most of the large members of the family Bovidae, having been recorded from only 27 species belonging to 17 genera in the family. It is interesting to note that he recorded *E. paradoxus* from nine antelopes, stating that the bushbuck was by far

the most common host, whilst the present survey shows that at least in some areas the common duiker can share this distinction. He stated that the latter was also frequently infested, and included the lesser kudu (*Tragelaphus* (*Strepsiceros*) *imberbia*) and greater kudu (*T.* (*S.*) *strepsiceros*). Reports of *E. paradoxus* from reedbuck, eland, waterbuck and impala he considered to be accidental infestations, although Meeser (1952) subsequently found that the impala was a fairly common host of this louse-fly in South Africa. Of the above-mentioned antelopes, it will be seen by reference to the host parasite list of *theileri*-like trypanosomes (table 2) that bushbuck and duiker in particular, and greater kudu, reedbuck, eland and waterbuck, have all been found infected. It is not suggested from these observations that *E. paradoxus* may be the only vector of *theileri*-like trypanosomes of antelopes, indeed it is quite possibly only one of a number of species of louse-flies which may act in this capacity. Nor is the possibility excluded of biting flies being vectors, especially as Bequaert (1942) stated that of the seven genera in the Bovidae from which no louse-flies of any genus had at that time been recorded, included the situtunga, which is known to be a host of *theileri*-like trypanosomes. Nevertheless, if the captive duikers in these investigations had become infested with hippoboscids, perhaps the results of blood examination would have been more revealing. On the other hand, the failure to find flagellates in 132 louse-flies (*Echestypus paradoxus*) could be interpreted as indicating that these hippoboscids are not vectors, especially as the infection rate of these insects is usually high for *T.* (*M.*) *melophagium* and *T.* (*M.*) *theodori*. In spite of these observations, until many more examinations of these ectoparasites have been made, they must be seriously considered as possible vectors of *theileri*-like trypanosomes of antelopes.

Summary of main conclusions

The present investigation has shown that *theileri*-like trypanosomes occur in at least 23 different species of ruminants, which with four exceptions belong to the family Bovidae. The parasites are particularly common in duikers in Central Africa and the highest incidence of infection occurs in the rainy season.

The duiker parasites are easily cultured on artificial media but are difficult to maintain.

The game trypanosomes appear to be more difficult to transmit by blood inoculation to other ruminants than are the cattle parasites, which according to some workers are relatively easily transmitted to other cattle by this means.

Although *theileri*-like trypanosomes cannot be separated into different species purely on the basis of morphological features using biometrical methods, it is possible, as stated by Herbert (1964), that different species do exist in addition to *T.* (*M.*) *theileri*, *T.* (*M.*) *melophagium* and *T.* (*M.*) *theodori*. Very little is known about their host relationships and methods of transmission, therefore future work on these subjects and on biochemistry and serology may well provide evidence of biological differences, enabling the creation of several other species as was originally attempted by early workers using only morphological variations. It is preferable at this stage not to include *T.* (*M.*) *ingens*, *T.* (*M.*) *tragelaphi* and *T.* (*M.*) *cephalophi* in the synonymy of the older species *T.* (*M.*) *theileri*, because this might lead to complications and confusion of nomenclature at a later date, when more research on biological characteristics has been carried out (Keymer 1967).

It seems likely that *theileri*-like trypanosomes of all ruminants multiply in the blood in the 'crithidial' (epimastigote) stage.

It is thought that hippoboscid flies of perhaps several species, and especially *E. paradoxus*, may be vectors of antelope *theileri*-like trypanosomes, in addition to biting flies and ticks.

(ii) *Other members of the family Trypanosomatidae*

In two separate areas of Rhodesia (habitats III*a* and *b*) where the tsetse fly *Glossina morsitans* was present, trypanosomes other than the *theileri*-type were found in the blood of two duikers. The level of parasitaemia was low in both antelopes, only two parasites being found in one individual and one in the other.

Description of parasites

It is impossible with the limited number of specimens available to identify the trypanosomes with certainty.

In the duiker from habitat III*b* the parasites measure 18·2 and 25·3 μm in total length. The former trypanosome has a prominent, virtually terminal kinetoplast, long free flagellum and a well-developed undulating membrane, denoting that it is probably *T. vivax*. In the other specimen, however, the kinetoplast is small, marginal and more subterminal than subcentral in position (according to the definitions of Hoare 1938). Although a long free flagellum is present, the undulating membrane is poorly developed. It is possible that this parasite is *T. brucei*, so that the duiker had a mixed infection. The single trypanosome in the animal from habitat III*a* also appears to be most likely *T. brucei*. It measures 18·8 μm in length, has a rather pointed posterior end with a small subterminal kinetoplast, short free flagellum and well-developed undulating membrane.

Other records of Trypanosoma *spp.*

During this survey all the previously recorded blood protozoa of the common duiker have been found, with the exception of some of the species of trypanosomes transmitted by *Glossina* spp. of tsetse-flies. This is probably because the majority of these antelopes were collected in areas where *Glossina* spp. were either absent or had been virtually eradicated by control measures.

Although Ashcroft (1959) did not list the duiker as an important reservoir host of trypanosomiasis, the main species have been recorded in this antelope. *T. vivax* and *T. congolense* were both reported by Kinghorn & Yorke (1912*b*) near Ngoa in N.E. Rhodesia (Zambia) and more recently da Silva (1959) has found duikers with the parasites in Mozambique. Other records of *T. congolense* include Vanderplank (1942) and Dias (1960), whilst Bruce *et al.* (1913*b*) found duikers infected with the *T. brucei* group in Nyasaland (Malawi) in the area where much of the present investigations have been carried out, but where tsetse-flies no longer exist.

(*c*) *Family Theileriidae*

Piroplasms: probably Cytauxzoon sylvicaprae

History and introduction

Piroplasms have been recorded before in the duiker on several occasions (Neitz 1957), and prior to 1948 were considered to belong to the genus *Theileria*. Neitz (1956), however,

revised the classification of piroplasms, retaining only the species *T. parva* in the genus *Theileria* in the family Theileriidae. In 1948, Neitz & Thomas created the genus *Cytauxzoon* and included it with the genus *Gonderia* in the family Gonderidae. The new genus was created by them for the new species *C. sylvicaprae*, this piroplasm having been found in the duiker in the Republic of South Africa. Neitz & Thomas (1948) removed the duiker parasite from the genus *Theileria* because they found schizogony in cells of the histiocytic series, whereas in *Theileria* schizogony is confined to cells of the lymphatic system. The intra-erythrocytic forms of *Theileria* and *Cytauxzoon* are said to be indistinguishable, and with the exception of *Theileria* spp. in the buffalo, Neitz (1964) believed that 'many, if not all' of the small piroplasms recorded in African wild ruminants may be *Cytauxzoon* spp. It is for this reason that the present parasite is regarded as being probably *C. sylvicaprae* in spite of the fact that no exo-erythrocytic stages have been found.

Not all protozoologists (e.g. Levine 1961) are convinced of the validity of the genus *Cytauxzoon* or its inclusion in the Gonderidae and prefer to retain piroplasms such as the duiker parasite in the family Theileriidae and genus *Theileria*. Brocklesby (1962) considered that in spite of the resemblances of the schizogonous stages of *Cytauxzoon*, *Hepatocystis* and *Leucocytozoon*, the genus *Cytauxzoon*, although valid, should be retained in the family Theileriidae because the erythrocytic forms and tick stages of *C. taurotragi*, the eland piroplasm are virtually indistinguishable from *T. parva*.

Although 110 duikers from seven different habitats were examined, piroplasms were found in only two animals representing an infection rate of 1·9 %. Both animals were shot in Zambia (habitat I*c*), where a total of 20 was examined making an infection rate there of 10 %, compared with an infection rate of 16·6 % recorded by Brocklesby & Vidler (1966) who examined six duikers shot in three different localities in Kenya.

Neitz & Thomas (1948) found schizonts of *C. sylvicaprae* closely resembling Koch's blue bodies of *T. parva* in smears of the spleen, liver, kidney and to a lesser extent the lungs. Neitz (1957) also reported schizonts in histiocytes in smears from the spleen, liver, lungs, kidneys and adrenals, but prolonged examinations of approximately 100 sections each of kidney and spleen and six splenic smears have failed to reveal the presence of exo-erythrocytic stages in the present material.

Description of parasites

The intra-erythrocytic stages which were found are illustrated in figure 30. The young forms (1) measure just over 1·0 × 0·5 μm and (2) 1·0 × 0·75 μm; ring forms (3) 1·0 × 1·0 and (4) 1·25 × 0·75 μm. The greatest dimensions of the irregular-shaped ring (5) are 1·5 × 1·0 and 2·0 × 1·25 μm. Comma-forms (7) are about 1·75 μm long and 1·0 μm across at the widest point, the nucleus being approximately 1·0 × 0·5 μm. The heart-shaped parasite which appears to be commencing binary fission (8) measures 1·75 × 1·5 μm in its greatest dimensions.

The illustrated forms are the only ones which were seen, the level of parasitaemia being low in both animals (5 parasites/1000 erythrocytes in no. 33 and 4/1000 in no. V.W. 13) with no multiple infections of the erythrocytes. All forms show a dark red-staining nucleus and faint blue-staining cytoplasm with Giemsa's stain. Morphologically the parasites are indistinguishable from the erythrocytic stages of *Cytauxzoon sylvicaprae* described by Neitz

& Thomas (1948), although no division into four was seen, resulting in 'cross forms' as described by Neitz (1964).

The vector of the duiker piroplasms is unknown, but likely to be a tick, although none was found on either of these infected antelopes. Ticks of at least five different species, however, were collected from duikers in the same habitat, namely, *Rhipicephalus* sp. near *Pravus*, other *Rhipicephalus* sp., *R. reichenowi*, *Ixodes* sp., *Hyalomma truncatum* and *Ablyomma variegatum*.

Levine (1961) listed ten species of ticks as vectors of the cattle parasite *T. parva*, the most important being *R. appendiculatus*, the only species included above which he mentioned being *H. truncatum*. The vectors of *C. taurotragi* are probably *R. appendiculatus* and *R. pulchellus* (Brocklesby 1962).

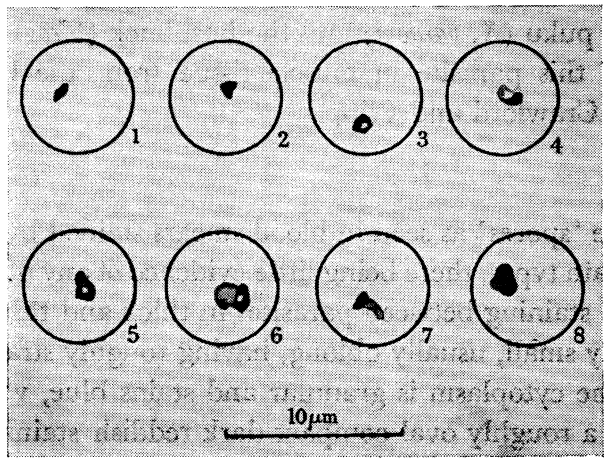


FIGURE 30. *Cytauxzoon sylvicapra* (or *Theileria* sp.) in the blood of a duiker (*Sylvicapra grimmia*). 1 and 2, young forms; 3 and 4, ring forms; 5 to 7, pleomorphic forms; 8, heart-shaped form which appears to be commencing binary fission.

(*d*) *Family Sarcocystidae*

Introduction

Although strictly speaking *Sarcocystis* species are not true blood protozoa, they have been included here because the 'spores' are sometimes found in blood smears. In this survey blood for examination has usually been taken from the heart with a syringe and needle. Undoubtedly the needle has sometimes ruptured cysts in the cardiac muscle and released the 'spores'. Similarly, cysts in skeletal muscle are ruptured when an animal is shot, resulting in the 'spores' being found in smears made from blood taken from shot wounds.

Sarcocystis 'spores' were found in blood smears from two duikers in Malawi (9·1 %, habitat II*a*) and six duikers in Rhodesia (12·5 %, habitat III*a*; 12·1 %, habitat III*b*). The higher incidence in Rhodesia may be apparent rather than real and indicate a higher incidence of infection in the skeletal than in the cardiac musculature, because the majority of blood smears from the Rhodesian duikers were taken from shot wounds, whilst in Malawi the heart blood was used.

In view of the relatively high incidence of the infection in these two different areas of Central Africa, it is surprising that there appear to be no other records of *Sarcocystis* in the duiker *S. grimmia*. The parasite is known to be virtually cosmopolitan and infects a wide range of vertebrates, domestic ruminants being frequently affected. It is probably also

common in wild ruminants and it has now been recorded in at least 11 species of wild Bovidae in Africa. In virtually all cases the description of the parasites are either meagre or non-existent, so that it is difficult to make useful comparisons between the present parasites of the duiker and those in other antelopes.

The first record in an African antelope appears to be that of Balfour (1913), who found *Sarcocystis* in the Korin gazelle (*Gazella rufifrons*). It has since been found by Dogiel (1915) in Coke's hartebeest (*Alcelaphus cokei*), and Grant's gazelle (*Gazella granti*), Fantham (1921) in the reedbuck (*Redunca arundinum*), Keymer (1963) in the waterbuck (*Kobus ellipsiprymnus*), Mandour (1964) in the Defassa waterbuck (*K. defassa*) and by Brocklesby & Vidler (1966) in the Uganda kob (*Adenota kob*) and wildebeest (*Connochaetes taurinus*).

Following further examination of blood smears collected by Keymer (1963) he later found *Sarcocystis* in the puku (*K. vardoni*) and the bushbuck (*Tragelaphus scriptus*). He also identified the cysts of this parasite in muscle tissue from the buffalo (*Syncerus caffer*) collected in Kenya by Crawford and Fripp.

Description of parasites

Morphologically, the 'spores' as seen in blood smears stained by Giemsa's method can be grouped into two main types, there being little evidence of any difference in appearance caused by fixation and staining between parasites in thick and thin blood smears.

One type is relatively small, usually oblong, having roughly straight and parallel sides with rounded ends. The cytoplasm is granular and stains blue, whilst the centre of the 'spore' is occupied by a roughly oval compact dark reddish staining nucleus (figure 31, (1) and (2); figure 32, plate 31). In some 'spores', however, the chromatin of the nucleus is not demonstrable and the body stains a dark bluish colour similar to that of the cytoplasm but only deeper. It can be seen from table 9, nos. 226, 44 and B56, that these parasites range from 4 to 12 μm in length and 1·75 to 4 μm in width.

The other types of 'spore' as illustrated in figure 31, (3) to (6) are larger and more typical of those described previously in various ruminants by other workers. The parasite usually appears as a sickle-shaped organism with round ends (3–5). Usually at least two-thirds of the 'spore' is occupied with bluish purple staining cytoplasm containing reddish or bluish purple granules. The granules may be fine or coarse in structure. Unlike the smaller 'spores' the nucleus is usually terminal in position, but occasionally central and stains a dark reddish colour. The chromatin is usually compact and dense (3, 4) although sometimes rather loose, showing strands or granules of darker staining and more compact chromatin (6). In a few 'spores' a second mass of chromatin is occasionally present, usually being central in position and sometimes shaped rather like a horse-shoe (4). This latter structure has been described previously, although its significance is unknown. These larger types of 'spore' usually measure about 15 μm in length, ranging from 12·5 to 21μm and in width from 3·0 to 8·5 μm (table 9, nos. 30, 31, 43, 57). It is interesting to note that in duiker no. 312 both types of 'spore' were seen (figure 31 (7, 8)).

Unfortunately skeletal or cardiac muscle was not available from the Rhodesian antelopes but prolonged examination of over 450 sections of both kinds of muscle from duikers shot in Malawi and careful observations during post-mortem examinations failed to reveal the presence of any cysts.

Discussion

Mandour (personal communication) found that the size of the 'spores' is not affected by the size of the cyst or the location of the parasite, and the present studies support the latter observation because both types of 'spore' were seen in blood from shot wounds and the heart. Mandour also believes that when *Sarcocystis* 'spores' from different hosts are compared, the measurements should be compared with either those in sections of cysts or in smears. If Mandour's opinions are subsequently shown to be correct and if the smaller type

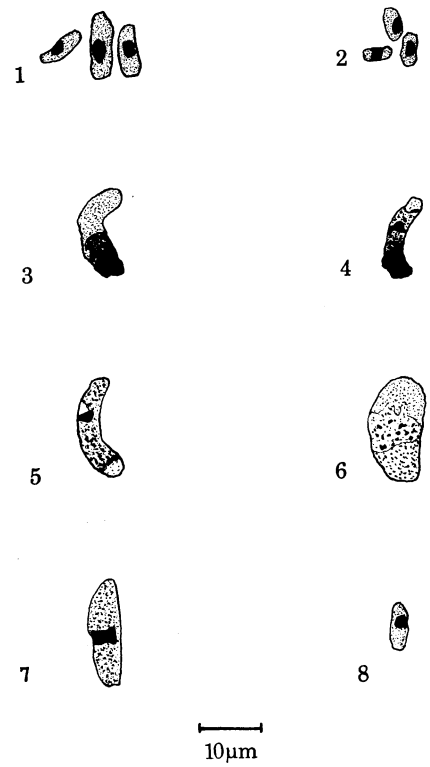


FIGURE 31. *Sarcocystis* 'spores' found in blood smears from duikers (*Sylvicapra grimmia*)

1, parasites from no. 44, showing compact nuclei which stain reddish with Giemsa's stain. The cytoplasm has a fine granular, dark blue appearance.

2, duiker no. B56. These 'spores' are similar to those in 1, but the dark areas stain blue.

3 and 4, from no. 30. In both parasites the cytoplasm is granular and purplish in colour, the nucleus being compact and dark red, whilst in 4 an additional, roughly horseshoe-shaped mass of chromatin is also present.

5, from no. 43. Immediately adjacent to the small reddish nucleus is a pale vacuolated area. At this end of the 'spore' the cytoplasm contains fine blue granules which extend to the other side of the nucleus and become larger in size and increasingly dark purple in colour, the background staining a pale blue.

6, from no. 57. The nucleus in this parasite is present as a pale pink irregularly shaped band extending across the middle of the 'spore'. Within the nucleus are darker pink staining granules. At one end of the 'spore' the purplish-blue cytoplasm is finely granular whilst at the other end the slightly larger granules stain a pale blue.

7 and 8, two types of 'spore' from no. 312. The larger 'spore' has a purplish-blue fine granular cytoplasm and a pale purplish-red nucleus. The other parasite is identical to those seen in duiker no. 44 (1).

TABLE 9. MEASUREMENTS OF *SARCOCYSTIS* 'SPORES' FOUND IN BLOOD SMEARS FROM DUIKERS (*SYLVICAPRA GRIMMIA*)

reference no.	habitat of host	source of blood sample	type of blood smear	no. measured	description	length (μm)		width (μm)	
						mean	range	mean	range
226/1	Malawi, no. II*a*	intracardiac	thick	1	'spore'	9·0	—	3·5	—
				1	nucleus	2·75	—	1·5	—
226/2			thin	10	'spore'	10·1	9·0–12·0	3·2	2·0–4·0
				10	nucleus	3·2	2·0–4·5	1·8	1·5–2·0
312	Malawi, no. II*a*	intracardiac	thin	5	'spore'	15·1	8·0–17·0	4·8	3·0–5·5
				5	nucleus	3·1	2·0–5·0	2·3	1·5–4·5
30	Rhodesia, no. III*a*	shot wound	thin	10	'spore'	15·0	12·5–18·0	3·5	3·0–5·0
				10	nucleus	5·75	3·5–8·0	3·5	2·5–5·0
31	Rhodesia, no. III*a*	shot wound	thick	1	'spore'	15·5	—	5·5	—
				1	nucleus	6·5	—	5·0	—
43	Rhodesia, no. III*a*	shot wound	thick	1	'spore'	17·5	—	4·0	—
				1	nucleus	4·5	—	3·0	—
43			thin	1	'spore'	21·0	—	4·0	—
				1	nucleus	2·5	—	1·5	—
44	Rhodesia, no. III*a*	shot wound	thin	10	'spore'	7·8	6·0–11·0	3·0	2·5–3·75
				10	nucleus	2·8	2·5–3·5	1·8	1·5–2·5
57	Rhodesia, no. III*a*	shot wound	thin	2	'spore'	15·75	14·5–17·0	7·5	6·5–8·5
				1	nucleus	6·5	—	4·5	—
B56	Rhodesia, no. III*b*	shot wound	thick	10	'spore'	5·0	4·0–6·0	2·25	1·75–2·75
				7	nucleus	2·25	2·0–2·5	1·75	1–2·5

of 'spores' described here are in fact *Sarcocystis* as believed and not *Besnoitia* or some other protozoan parasite, then it is quite likely that they belong to a new species. Whether or not the two types of 'spores' represent one or two species of the parasite or different degrees of maturity of one parasite it is impossible to state, in the absence of any cysts being found in the musculature. The larger 'spores' are typical of *Sarcocystis* but the smaller organisms do not appear to have been described previously and it is possible therefore that they may represent another protozoan parasite.

I am deeply indebted to Professor P. C. C. Garnham, C.M.G., F.R.S., Department of Parasitology, London School of Hygiene and Tropical Medicine, for constant advice and encouragement, and without whose assistance this research could not have been done. Drs W. E. Ormerod and R. S. Bray of the same Department also gave me helpful advice and especially Dr C. A. Hoare, F.R.S., of the Wellcome Trust. I am grateful to Professor R. D. Remington for advice with statistical problems and to Messrs J. Nesbit, R. Killick-Kendrick and Mrs R. Page for technical assistance. Dr B. R. Laurence of the Entomology Department identified the hippoboscids and Dr H. Hoogstraal, U.S. Naval Medical Research Unit, Cairo, Egypt, identified the ticks.

Many people helped me in Africa and unfortunately it is not possible to mention them all by name. The following persons in particular, however, provided assistance in various ways; Messrs V. J. Wilson (Zambia) and A. R. Tribe (Malawi) helped me to obtain live duikers; Mr P. Bannister, Director of the Department of Veterinary Services in Malawi, provided transport, whilst his staff and Mr P. Hanney, Curator of the Nyasaland Museum, assisted with laboratory equipment and in other ways; Mr Peter Brown, Senior Agricultural Officer, provided facilities and accommodation at the Chitala Experimental Station, Malawi; Messrs D. Fairhall and D. Mansfield assisted with hunting and the former also with surgery; Mr W. E. Grainger provided technical assistance with the entomological work; and my African staff, especially Mr Santoni Pirie, helped in a multitude of ways.

Dr R. Roth, Department of Veterinary Services (Trypanosomiasis), Rhodesia, kindly made available for study a large collection of blood smears from duikers.

Dr J. Brooksby, the Director of the Animal Virus Research Institute, and Mr D. A. Barr at Pirbright, Surrey, provided facilities in this country for housing and examining the blood of splenectomized ungulates, whilst Mr S. F. M. Davies and Dr L. P. Joyner at the Central Veterinary Laboratory, Weybridge, kindly splenectomized the three ruminants which were housed at Pirbright.

I am grateful to the Royal Society of Tropical Medicine and Hygiene for allowing me to study the original water-colour drawings of duiker blood protozoa painted by Lady Bruce for her husband, Sir David Bruce, when they were working in Nyasaland during the early years of this century.

Finally, Mr C. J. Webb, London School of Hygiene and Tropical Medicine, and Mr T. Dennett, Zoological Society of London, helped with the photography and the preparation of the illustrations.

I am indebted to the Ministry of Overseas Development for financial assistance.

References

Ansell, W. F. H. 1960 *Mammals of Northern Rhodesia.* Lusaka, Northern Rhodesia: The Government Printer.

Ansell, W. F. H. 1963 Additional breeding data on Northern Rhodesian mammals. *The Puku. Occasional Papers Department Game and Fisheries, N. Rhodesia,* no. 1, pp. 9–28.

Ashcroft, M. T. 1959 The importance of African wild animals as reservoirs of trypanosomiasis. *E. Afr. med. J.* **36**, 289–297.

Bailey, N. T. J. 1960 *Statistical methods in biology.* London: The English University Press Ltd.

Baker, J. R. 1956 Studies on *Trypanosoma avium* Danilewsky 1885. II. Its transmission by *Ornithomyia avicularia* L. *Parasitology* **46**, 321–334.

Balfour, A. 1913 A sarcocyst of a gazelle (*G. rufifrons*) showing differentiation of spores by vital staining. *Parasitology* **6**, 52–56.

Behn, P. 1910 Infektion eines Kalbes mit Trypanosomen vom Typus des *Trypanosoma theileri* mittelst Blut von Kühen, in denen nur kulturell Flagellaten nachweisbar waren. *Berl. tierärztl. Wschr.* **26**, 998–1000.

Bequaert, J. 1942 A monograph of the Melophaginae, or ked-flies, of sheep, goats, deer and antelopes (Diptera, Hippoboscidae). *Entomologica am.* **22** (N.S.), 1–220.

Best, G. A., Edmond-Blanc, F., Witting, R. C. & Raw, W. G. 1962 *Rowland Ward's records of big game,* 11th ed. (African.) London: Rowland Ward.

Brass, J. 1953 Vegetation of Nyasaland. Report of the Vernay Nyasaland Expedition of 1946. *Mem. N.Y. Bot. Gdn* **8**, 161–190.

Brocklesby, D. W. 1962 *Cytauxzoon taurotragi* Martin and Brocklesby 1960, a piroplasm of the eland (*Taurotragus oryx pattersonianus* Lydekker, 1906). *Res. vet. Sci.* **3**, 334–344.

Brocklesby, D. W. & Vidler, B. O. 1965 Some parasites of East African wild animals. *E. Afr. Wildlife J.* **3**, 120–122.

Brocklesby, D. W. & Vidler, B. O. 1966 *Haematozoa* found in wild members of the order Artiodactyla in East Africa. *Bull. epizoot. Dis. Afr.* **14**, 285–299.

Bruce, D. 1902 Note on the discovery of a new trypanosome. *Lancet* **1**, 664.

Bruce, D., Hamerton, A. E. & Bateman, H. R. 1909*a* A trypanosome from Zanzibar. *Proc. Roy. Soc.* B **81**, 14–30.

Bruce, D., Hamerton, A. E., Bateman, H. R. & Mackie, F. P. 1909*b* *Trypanosoma ingens* n.sp. *Proc. Roy. Soc.* B **81**, 323.

Bruce, D., Hamerton, A. E., Bateman, H. R., Mackie, F. P. & Lady Bruce, R. R. C. 1911*a* Appendix II. Report on an outbreak of disease among cattle on the Namukekera estate belonging to the Uganda Company Limited. *Rep. Sleep. Sickn. Comm. Roy. Soc.* **11**, 260–264.

Bruce, D., Hamerton, A. E., Bateman, H. R., Mackie, F. P. & Lady Bruce, R. R. C. 1911*b* 10. Experiments to ascertain if antelope may act as a reservoir of the virus of sleeping sickness (*Trypanosoma gambiense*). *Rep. Sleep. Sickn. Comm. Roy. Soc.* **11**, 75–92.

Bruce, D., Harvey, D., Hamerton, A. E. & Lady Bruce, R. R. C. 1913*a* *Plasmodium cephalophi* sp.nov. *Proc. Roy. Soc.* B **87**, 45–47.

Bruce, D., Harvey, D., Hamerton, A. E., Davey, J. B. & Lady Bruce, R. R. C. 1913*b* The trypanosomes found in the blood of wild animals living in the sleeping-sickness area, Nyasaland. *Proc. Roy. Soc.* B **86**, 269–284, and also in *Rep. Sleep. Sickn. Comm. Roy. Soc.* (1914), **15**, 16–24.

Bruce, D., Harvey, D., Hamerton, A. E., Davey, J. B. & Lady Bruce, R. R. C. 1914 Trypanosome diseases of domestic animals in Nyasaland. II. *Trypanosoma caprae* (Kleine). *Rep. Sleep. Sickn. Comm. Roy. Soc.* **15**, 25–31.

Bruce, D., Harvey, D., Hamerton, A. E., Davey, J. B. & Lady Bruce, R. R. C. 1915 Trypanosomes and other parasites of animals in Nyasaland. *Rep. Sleep. Sickn. Comm. Roy. Soc.* **16**, 203–206.

Carmichael, J. 1939 Turning sickness of cattle and *Trypanosoma theileri. Parasitology* **31**, 498–500.

Carpano, M. 1932 Localisations du *Trypanosoma theileri* dans les organes internes des bovins. Son cycle évolutif. *Ann. Parasit. hum. comp.* **10**, 305–322.

Cater, J. C., 1954 The Nyika Plateau, Nyasaland. *Oryx* **2**, 298–302.

Chambers, E. G. 1943 *Statistical calculation for beginners.* Cambridge University Press.

Child, G. & Wilson, V. J. 1964*a* Delayed effects of tsetse control hunting on a duiker population. *J. Wildlife Mgmt* **28**, 866–868.

Child, G. & Wilson, V. J. 1964*b* Observations on ecology and behaviour of Roan and Sable in three Tsetse Control Areas. *Arnoldia* **1**, 1–8.

Curasson, C. 1925 *Trypanosoma theileri* au Soudan francais. *Bull. Soc. Pat. exot.* **18**, 823–824.

Curasson, C. 1943 *Traité de protozoologie vétérinaire et comparée.* Vol. 1, *Trypanosomes*, p. 235. Paris: Vigot Frères.

Davis, B. S. 1952 Studies on the trypanosomes of some California mammals. *Univ. Calif. Publ. Zool.* **57**, 145–200.

Deane, L. M. 1961 Tripanosomideros de Mamiferos de Reĝiao Amazonica. *Rev. Inst. Med. trop. S. Paulo* **3**, 15–28.

Dias, J. A. T. S. 1960 Trypanosomes found in antelopes of a region infested by *Glossina austeni* Newstead 1912 in Mozambique. *8th Meet. Int. Sci. Comm. Tryp. Res.* **7**, 175–177. (Published under the sponsorship of the Commission for Technical Cooperation in Africa, South of the Sahara.)

Dios, R. K. & Zuccarini, J. A. 1924 Première constation de la trypanosomiase bovine en republique Argentine. *C.R. Soc. Biol., Paris* **91**, 23–24.

Dodd, S. 1912 *Trypanosoma ingens* in the mouse deer (*Tragulus javanicus*). *J. comp. Path.* **25**, 281–285.

Dogiel, V. A. 1915 Two new species of *Sarcocystis* from African antelopes, part 8, pp. 10–13. In *Scientific Results of the Zoological Expedition to British East Africa and Uganda made by Professor V. Dogiel and J. Sokolow in the year* 1914, vol. 1. Petrograd.

Duke, H. L. 1912 Antelope and their relation to trypanosomiasis. *Proc. Roy. Soc.* B **85**, 156–169.

Dutton, J. E., Todd, J. L. & Tobey, E. N. 1906 Concerning certain parasitic protozoa observed in Africa. Part 1. *Liverpool Sch. Trop. Med. Mem.* **21**, 87–97.

Ellerman, J. R., Morrison-Scott, T. C. S. & Hayman, R. W. 1953 *Southern African Mammals* 1758–1951: *A reclassification.* Printed by order of the Trustees of the British Museum, London.

Fairbairn, H. 1953 Studies on *Trypanosoma vivax.* IX. Morphological differences in strains and their relation to pathogenicity. *Ann. trop. Med. Parasit.* **47**, 394–405.

Fairbairn, H. 1962 Measurements of strains of *Trypanosoma congolense. Ann. trop. Med. Parasit.* **56**, 218–221.

Fantham, H. B. 1921 Some parasitic protozoa found in South Africa. IV. *S. Afr. J. Sci.* **18**, 164–170.

Flu, P. C. 1908 Über die Flagellaten in Darm von *Melophagus ovinus. Arch. Protistenk.* **12**, 147. (Quoted by Neveau-Lemaire, 1943.)

Fraser, A. D. & Duke, H. L. 1912 The relation of wild animals to trypanosomiasis. A. Examination of wild animals with a view of ascertaining whether they are naturally infected with Trypanosomiasis. *Rep. Sleep. Sickn. Comm. Roy. Soc.* **12**, 26–36.

Galliard, H. 1925 Sur un cas d'infection a *Trypanosoma theileri* et *Piroplasma bigeminum. Ann. Parasit. hum. comp.* **3**, 21–27.

Garnham, P. C. C. 1964 The subgenera of *Plasmodium* in mammals. *Ann. Soc. belge Med. trop.* **44** 267–272.

Garnham, P. C. C. 1966 *Malaria parasites and other Haemosporidia*, pp. 486–490. Oxford: Blackwell.

Gates, D. W. 1953 The technique employed in splenectomy of bovine animals. *J. Am. Vet. med. Ass.* **122**, 94–98.

Godfrey, D. G. 1960 Types of *T. congolense.* I. Morphological differences. *Ann. trop. Med. Parasit.* **54**, 428–438.

Godfrey, D. G. 1961 Types of *Trypanosoma congolense.* II. Differences in the courses of infection. *Ann. trop. Med. Parasit.* **55**, 154–166.

Hamerton, A. E. 1911 An experiment to ascertain at what point on the road between Kavirondo and Uganda, cattle become infected with trypanosomes. An account of a journey from Kisumu to Jinja. *Rep. Sleep. Sickn. Comm. Roy. Soc.* **11**, 271–275.

Haughwont, F. G. & Youngberg, S. 1920 A trypanosome associated with a fatal disease in the carabao. *Phillip. J. Sci.* **16**, 77–87.

Herbert, I. V. 1964 *Trypanosoma theileri* Laveran, 1902. A cosmopolitan parasite of cattle. *Vet. Bull.* **34**, 563–570.

Herbert, I. V. 1965 Some observations on the isolation and *in vitro* culture of two mammalian trypanosomes, *Trypanosoma theileri* Laveran, 1902, and *T. melophagium* Flu, 1908, with special reference to *T. theileri*. *Ann. trop. Med. Parasit.* **59**, 277–293.

Hoare, C. A. 1923 An experimental study of the sheep trypanosome (*T. melophagium* Flu, 1908) and its transmission by the sheep ked (*Melophagus ovinus* L.). *Parasitology* **15**, 365–424.

Hoare, C. A. 1931 Studies on *Trypanosoma grayi*. III. Life-cycle in the tsetse-fly and in the crocodile. *Parasitology* **23**, 449–484.

Hoare, C. A. 1936*a* Morphological and taxonomic studies on mammalian trypanosomes. II. *Trypanosoma simiae* and acute porcine trypanosomiasis in tropical Africa. *Trans. R. Soc. trop. Med. Hyg.* **29**, 619–645.

Hoare, C. A. 1936*b* Morphological and taxonomic studies on mammalian trypanosomes. I. Method of reproduction in its bearing upon classification, with special reference to the *Lewisi* group. *Parasitology* **28**, 98–109.

Hoare, C. A. 1938 Morphological and taxonomic studies on mammalian trypanosomes. V. The diagnostic value of the kinetoplast. *Trans. R. Soc. trop. Med. Hyg.* **32**, 333–342.

Hoare, C. A. 1949 *Handbook of Medical Protozoology*, p. 217. London: Baillière, Tindall and Cox.

Hoare, C. A. 1956 Morphological and taxonomic studies on mammalian trypanosomes. VIII. Revision of *Trypanosoma evansi*. *Parasitology* **46**, 130–172.

Hoare, C. A. 1959 Morphological and taxonomic studies on mammalian trypanosomes. IX. Revision of *Trypanosoma dimorphon*. *Parasitology* **49**, 210–231.

Hoare, C. A. 1966 The classification of mammalian trypanosomes. *Ergebn. Mikrobiol. Immunitäts-forsch.* **39**, 43–57.

Hoare, C. A. & Broom, J. C. 1938 Morphological and taxonomic studies on mammalian trypanosomes. IV. Biometrical study of the relationship between *Trypanosoma uniforme* and *T. vivax*. *Trans. R. Soc. trop. Med. Hyg.* **31**, 517–534.

Hoare, C. A. & Wallace, F. G. 1966 Developmental stages of trypanosomatid flagellates: a new terminology. *Nature, Lond.* **212**, 1385–1386.

Hoogstraal, H. 1956 Faunal exploration as a basic approach for studying infections common to man and animals. *E. Afr. med. J.* **33**, 417–424.

Ingles, J. M. 1965 Zambian mammals collected for the British Museum (Natural History). *The Puku. The Occasional Papers of the Department of Game and Fisheries, Zambia*, no. 3, pp. 75–86.

Kenneth, J. H. & Ritchie, G. R. 1953 *Gestation periods, a table and bibliography*, 3rd ed., p. 10. Technical Communications no. 5 of the Commonwealth Bureau of Animal Breeding and Genetics, Edinburgh. Commonwealth Agricultural Bureau, Farnham Royal, Slough, Bucks.

Keymer, I. F. 1963 Blood protozoa of wild mammals in Northern Rhodesia. Abstract 103, *J. Protozool.* **10** (Supplement), p. 30.

Keymer, I. F. 1966 Studies on *Plasmodium* (*Vinckeia*) *cephalophi* of the grey duiker (*Sylvicapra grimmia*). *Ann. trop. Med. Parasit.* **60**, 129–138.

Keymer, I. F. 1967 *Trypanosoma* (*Megatrypanum*) spp. Hoare, 1964, of ruminants. *Vet. Rec.* **80**, 286.

Kinghorn, A. & Yorke, W. 1912*a* Trypanosomes infecting game and domestic stock in the Luangwa valley, north eastern Rhodesia. *Ann. trop. Med. Parasit.* **6**, 301–315.

Kinghorn, A. & Yorke, W. 1912*b* Further observations on the trypanosomes of game and domestic stock in north eastern Rhodesia. *Ann. trop. Med. Parasit.* **6**, 483–489.

Kinghorn, A., Yorke, W. & Lloyd, L. 1913 Final report of the Luangwa Sleeping Sickness Commission of the British South Africa Company 1911–1912. Description of Trypanosomes. *Trypanosoma tragelaphi* sp.nov. *Ann. trop. Med. Parasit.* **7**, 269.

Kleine, F. K. & Fischer, W. 1912 Die Rolle der Säugetiere bei der Verbreitung der Schlafkrankheit und Trypanosomenbefunde bei Säugetieren am Tanganyka. *Z. Hyg. Infekt Kr.* **70**, 1–23. (Quoted by Wenyon 1926.)

Kraneveld, F. C. 1931 Einzelne Uebertragungs-versuche von *Trypanosoma theileri* Laveran 1902 mit Tabaniden (Vorläu-foge Mitterlung). *Nederl. Indishe Blad. Diergeneesk* **43**, 132–192.

Landau, I. & Killick-Kendrick, R. 1966 Rodent plasmodia of the Republique Centrafricaine: the sporogony and tissue stages of *Plasmodium Chabaudi* and *P. Berghei Yoelii*. *Trans. R. Soc. trop. Med. Hyg.* **60**, 633–649.

Laveran, A. 1902 Sur un nouveau trypanosome des Bovidés. *C.R. Acad. Sci., Paris* **134**, 512–514.

Laveran, A. 1903 Au sujet de deux trypanosomes des Bovidés du Transvaal. *C.R. Acad. Sci., Paris* **135**, 717–721.

Laveran, A. & Mesnil, F. 1912 *Trypanosomes et trypanosomiases*, 2nd ed. (See coloured plate at end of book.) Paris: Masson et C^{ie}.

Levine, N. D. 1961 *Protozoan parasites of domestic animals and man.* Minnesota: Burgess Pub. Co.

Levine, N. D., Watrach, A. M., Kantor, S. & Hardenbrook, H. J. 1956 A case of bovine trypanosomiasis due to *Trypanosoma theileri* in Illinois. *J. Parasit.* **42**, 553.

Linzell, J. L. 1964 General and regional anaesthesia in goats. In *Small animal anaesthesia. Proceedings of a symposium organised by the B.S.A.V.A. and U.F.A.W., London, July* 1963, pp. 163–175. Oxford: Pergamon Press.

Luhs, F. 1906 *Trypanosoma theileri* in Transkaukasien. *Arch. Parasit.* **10**, 175–186.

Lydekker, R. 1926 *The game animals of Africa*, 2nd ed., revised by J. G. Dollman. London: Rowland Ward Ltd.

Mackie, F. P. 1911 Appendix III. Report on an outbreak of disease among cattle on the estate of A. B. Walsh Esq., at Kabula Muliro. *Rep. Sleep. Sickn. Comm. Roy. Soc.* **11**, 264–268.

Mandour, A. M. 1964 A report on the presence of *Sarcocystis* in different hosts. *Trans. R. Soc. trop. Med. Hyg.* **58**, 287–288.

Mazza, S., Romaña, C. & Fiora, A. 1932 Algunos hemoparásitos de mamiferos delnorte. VII. *Reun. Soc. Argent. Patol. Reg. Norte* (*Buenos Aires*), part 2, pp. 990–997.

Meeser, M. J. N. 1952 A preliminary survey of the endo- and ectoparasites of the impala—*Aepyceros melampus*. *J. S. Afr. vet. med. Ass.* **23**, 221–223.

Morris, D. 1965 *The mammals. A guide to the living species.* London: Hodder and Stoughton.

Neitz, W. O. 1956 Classification, transmission and biology of piroplasms of domestic animals. *Ann. N.Y. Acad. Sci.* **64**, 56–111.

Neitz, W. O. 1957 Theileriosis, gonderioses and cytauxzoonoses. A review. *Onderstepoort J. vet. Res.* **27**, 275–430.

Neitz, W. O. 1964 Cytauxzoonoses in African antelopes. *Bull. off. int. Epiz.* **62**, 1009–1015.

Neitz, W. O. & Thomas, A. D. 1948 *Cytauxzoon sylvicaprae* gen.nov., spec.nov., a protozoon responsible for a hitherto undescribed disease in the duiker (*Sylvicapra grimmia* (Linné)). *Onderstepoort J. vet. Sci. Anim. Ind.* **23**, 63–76.

Neveu-Lemaire, M. 1943 *Traité de protozoologie médicale et vétérinaire*, p. 807. Paris: Vigot Frères.

Nicolle, C. 1907 Sur une piroplasme nouvelle d'un rongeur. *C.R. Soc. Biol., Paris* **63**, 213–216.

Nöller, W. 1916 Die übertrugung des *Trypanosoma theileri* Laveran, 1902 (Vorl. Mitt). *Berl. tierärztl. Wsch.* **32**, 457–460.

Nöller, W. 1917 Blut- und Insekten-flagellatenzuchtung auf Platten. *Arch. Schiffs- u. Tropenhyg.* **21**, 53. (Quoted by Wenyon 1926.)

Nöller, W. 1925 Der Nachweis des überträgers des gemeinen Rindertrypanosomas. *Trypanosoma theileri* mit Hilfe des Kulturverfahrens. *Zbl. Bakt.* I, **79**, 113–142.

O'Farrell, W. R. 1913 Hereditary infection with special reference to its occurrence in *Hyalomma aegyptium* infected with *Crithidia hyalommae*. *Ann. trop. Med. Parasit.* **7**, 545–562.

Old, J. E. S. 1915 Appendix III. Report on certain investigations in the elephant marsh; Ruo district. *Rep. Sleep. Sickn. Comm. Roy. Soc.* **16**, 210–212.

Pease, H. T. 1909 *Trypanosoma theileri* (Laveran) and Galziekte. *J. trop. vet. Sci.* **4**, 532–539.

Peter, O. 1910 Morpholgische und experimentalle Studien über ein neues bei Rindern in Uraguay (Sudamerika) gefundened Trypanosoma. Bei Lefts (6). *Arch. Schiffs-u. Tropenhyg.* **14**, 7–40. (Quoted by Galliard 1925.)

Pfeiffer, E. 1905 Über trypanosomenahnliche Flagellaten in Darm von *Melophagus ovinus*. *Z. Hyg. Infekt Kr.* **50**, 324. (Quoted by Neveu-Lemaire 1943.)

Potter, B. J. 1958 Haemoglobinuria caused by propylene glycol in sheep. *Brit. J. Pharmac.* **13**, 385–389.

Reichenow, E. 1940 Ostafrikanische Beobachtungen an Trypanosomiden. *Arch. Protistenk.* **94**, 267–287.

Richardson, U. F. & Kendall, S. B. 1963 *Veterinary protozoology*, p. 61. Edinburgh: Oliver and Boyd.

Riney, T. & Child, G. 1960 Breeding season and the ageing criteria for the common duiker (*Sylvicapra grimmia*). *Proc. 1st Fed. Sci. Congr.* pp. 291–299.

Ristic, M. & Trager, W. 1958 Cultivation at 37 °C of a trypanosome (*Trypanosoma theileri*) from cows with depressed milk production. *J. Protozool.* **5**, 146–148.

Roberts, A. 1951 *The mammals of South Africa*. Johannesburg: Trustees of 'The Mammals of South Africa' Book Fund.

Robinson, G. 1929 *Report of the Medical and Sanitary Department of the Gold Coast* 1928–1929, pp. 76, 77.

Rodhain, J. 1916 Note sur les trypanoses et les piroplasmoses des grands animaux de l'Ouellé. *Bull. Soc. Pat. exot.* **9**, 95–109.

Rodhain, J., Pons, C., Van Den Branden, F. & Bequaert, J. 1913*a*. Notes sur quelques hématozoaires du Congo belge. *Arch. Protistenk.* **29**, 260–261.

Rodhain, J., Pons, C., Van Den Branden, F. & Bequaert, J. 1913*b* *Rapport sur les travaux de la Mission Scientifique du Katanga* (*Octobre* 1910 *à Septembre* 1912). Bruxelles: Royaume de Belgique — Ministère des Colonies, pp. 139–150.

Roth, H. H. & Dalchow, W. 1967 Untersuchungen über den Wurmbefall von Antilopen in Rhodesien. *Z. angew. Zool.* **54**, 203–226.

Saisawa, K., Taise, K. & Kaneko, K. 1933 Études sur *Tr. theileri*. *Jap. J. exp. Med.* **11**, 101–105.

Schein, H. E. E. 1907 Hematozoaires des bovides en Indo-Chine. *Ann. Inst. Pasteur* **21**, 659–665.

Schwetz, J. 1930 Sur un cas d'infection intense par *Trypanosoma theileri*. *Ann. Parasit. hum. comp.* **8**, 135–139.

Schwetz, J. 1931 Notes morphologiques sur les trypanosomoses animales de Stanleyville et du Congo Oriental. *Ann. Parasit. hum. comp.*, **9** 392–422.

Sergent, Ed. & Sergent, Et. 1911 Présence de trypanosomes chez les bovidés en Algérie. *Bull. Soc. Pat. exot.* **4**, 40–42.

Shaw, J. J. 1964 Studies on members of the Trypanosomatidae in sloths, with special reference to *Endotrypanum schaudinni*. A thesis submitted for the degree of Doctor of Philosophy (Faculty of Science) of the University of London, July 1964, pp. 297–298.

Ben Shaul, D. M. 1963 The composition of the milk of wild animals. In *The International Zoo Yearbook* **4**, 333–342.

Shortridge, G. C. 1934 *The mammals of South-West Africa*, 2 vols. London: Heinemann.

da Silva, J. M. 1959 Notes sur l'étude de la trypanosomiase animale au Mozambique. *Bull. epiz. Dis. Afr.* **7**, 137–147.

Simpson, G. G. 1945 The principles of classification and a classification of mammals. *Bull. Am. Mus. Nat. Hist.* **85**, 157–162.

Southern, H. N. & Thompson, H. V. 1964 *In the handbook of British mammals*, p. 254. Oxford: Blackwell.

Stephen, L. E. 1963 A morphological and biometrical study of the blood forms of *Trypanosoma suis* Ochmann, 1905. *Parasitology* **53**, 217–223.

Stevenson-Hamilton, J. 1947 *Wild Life in South Africa*. London: Cassel.

Stockman, S. 1910 Preliminary note on a trypanosome of British cattle. *J. comp. Path.* **23**, 189–192.

Taliaferro, W. H. 1923 A study of size and variability throughout the course of 'pure line' infections, with *Trypanosoma lewisi*. *J. exp. Zoo.* **37**, 127–168.

Taliaferro, W. H. 1926 Variability and inheritance of size in *Trypanosoma lewisi*. *J. exp. Zool.* **43**, 429–473.

Theiler, A. 1903 A new trypanosome and the disease caused by it. *J. comp. Path.* **16**, 193–216.

Theodor, O. 1928 On the occurrence of a non-pathogenic trypanosome in the goat and its transmission by *Lipoptena caprina* Austen. (Preliminary note.) *Trans. R. Soc. trop. Med. Hyg.* **21**, 489–490.

Theodor, O. 1929 Über ein nicht pathogenes *Trypanosoma* aus der Ziege un seine Übertragung durch *Lipoptena caprina* Austen. *Z. Parasitenk.* **1**, 283–330.

Thomas, A. D. & Neitz, W. O. 1933 The importance of disease in wild animals. *S. Afr. J. Sci.* **30**, 419–425.

Thomson, J. G. 1931 *Trypanosoma tragelaphi* (Kinghorn, Yorke and Lloyd 1913) from the blood of the humped zebu (*Bos indicus*) in Nigeria. *Trans. R. Soc. trop. Med. Hyg.* **25**, 5–6.

Turner, A. W. & Murnane, D. 1930*a* On the presence of the non-pathogenic *Trypanosoma melophagium* in the blood of Victorian sheep and its transmission by *Melophagus ovinus*. *Aust. J. exp. Biol. med. Sci.* **7**, 5–8.

Turner, A. W. & Murnane, D. 1930*b* A preliminary note on the occurrence of *Trypanosoma theileri* in the blood of cattle in Victoria. *Aust. J. exp. Biol. med. Sci.* **7**, 9–11.

Vanderplank, F. L. 1942 A note on trypanosomiasis of game from tsetse areas at Shinyanga and Ukerwe Peninsula. *Trans. R. Soc. trop. Med. Hyg.* **35**, 319–322.

Vivier, E. & Schrevel, J. 1964 Étude, au microscope électronique, d'une grégarine du genre *Selenidium* parasite de *Salbeelaria alveolata* L. *J. Microsc., Paris*, **3**, 651–670.

Walker, P. J. 1963 Variation in the length of trypanosomes after different methods of staining. *Trans. R. Soc. trop. Med. Hyg.*, **57**, 237.

Wallace, F. G. 1962 The Trypanosomatid parasites of horseflies with the description of *Crithidia rileyi* n.sp. *J. Protozool.* **9**, 53–58.

Wells, E. A., Lumsden, W. H. R., Hardy, G. J. C. & James, D. 1965 Observations of *Trypanosoma theileri* Laveran in the United Kingdom and in Nigeria. Abstract no. 158, p. 145. *Progress in Protozoology*. Abstracts of papers read at 2nd International Conference on Protozoology. International Congress Series, no. 91. London: Excepta Medica Foundation.

Wenyon, C. M. 1926 *Protozoology. A manual for medical men, veterinarians and zoologists.*, 2 vols. London: Baillière, Tindall and Cox.

Wilson, V. J. 1965 Observations on the greater kudu *Tragelaphus strepsiceros* Pallas from a tsetse control hunting scheme in Northern Rhodesia. *E. Afr. Wildl. J.* **3**, 27–37.

Wilson, V. J. & Child, G. F. T. 1964 Notes on Bushbuck (*Tragelaphus scriptus*) from a tsetse fly-control area in Northern Rhodesia. *The Puku. The Occasional Papers of the Department of Game and Fisheries, Zambia*, no. 2, pp. 118–128.

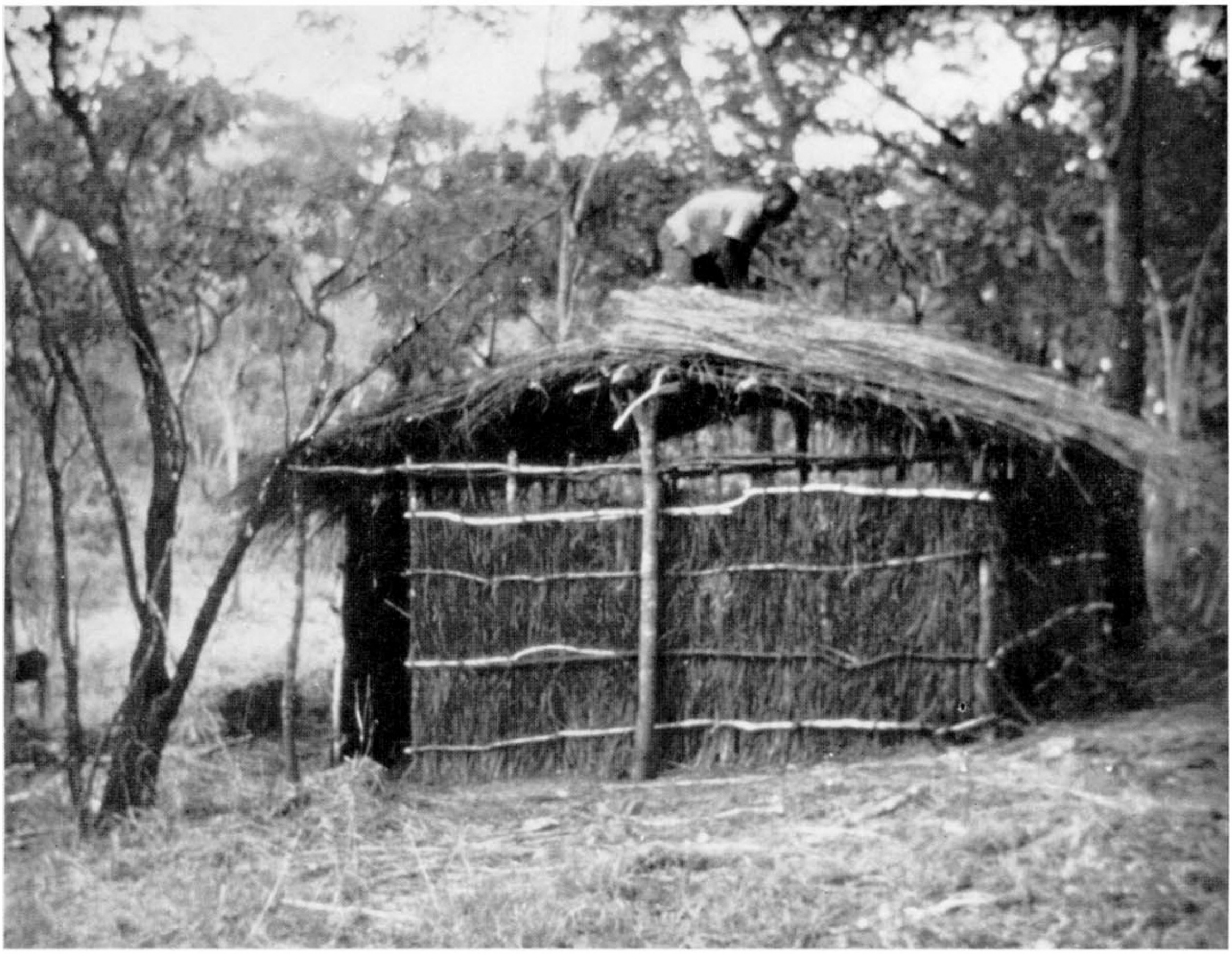
Wilson, V. J. & Clarke, J. E. 1962 Observations on the common duiker *Sylvicapra grimmia* Linn., based on material collected from a tsetse control game elimination scheme. *Proc. zool. Soc. Lond.* **138**, 487–497.

Wright, J. G. & Hall, L. W. 1961 *Veterinary anaesthesia and analgesia*, 5th ed. London: Baillière, Tindall and Cox.

Wrublewsky, K. I. 1908 Trypanosoma des Wisent (*Bonasus bison*) im Bielowescher Walde. *Zbl. Bakteriol* (1. Abt.), **41**, 24–25. (Quoted by Wenyon 1926.)

Yaeger, R. G. 1960 A method of isolating trypanosomes from blood. (Research note.) *J. Parasit.* **46**, 288.

Yakimoff, W. L., Nezwetajeff, N. W. & Petunina, P. I. 1933 Les parasites du sang des zébus. *Berl. Münch. tierärztl. Wschr.* no. 52, pp. 824–827.



2

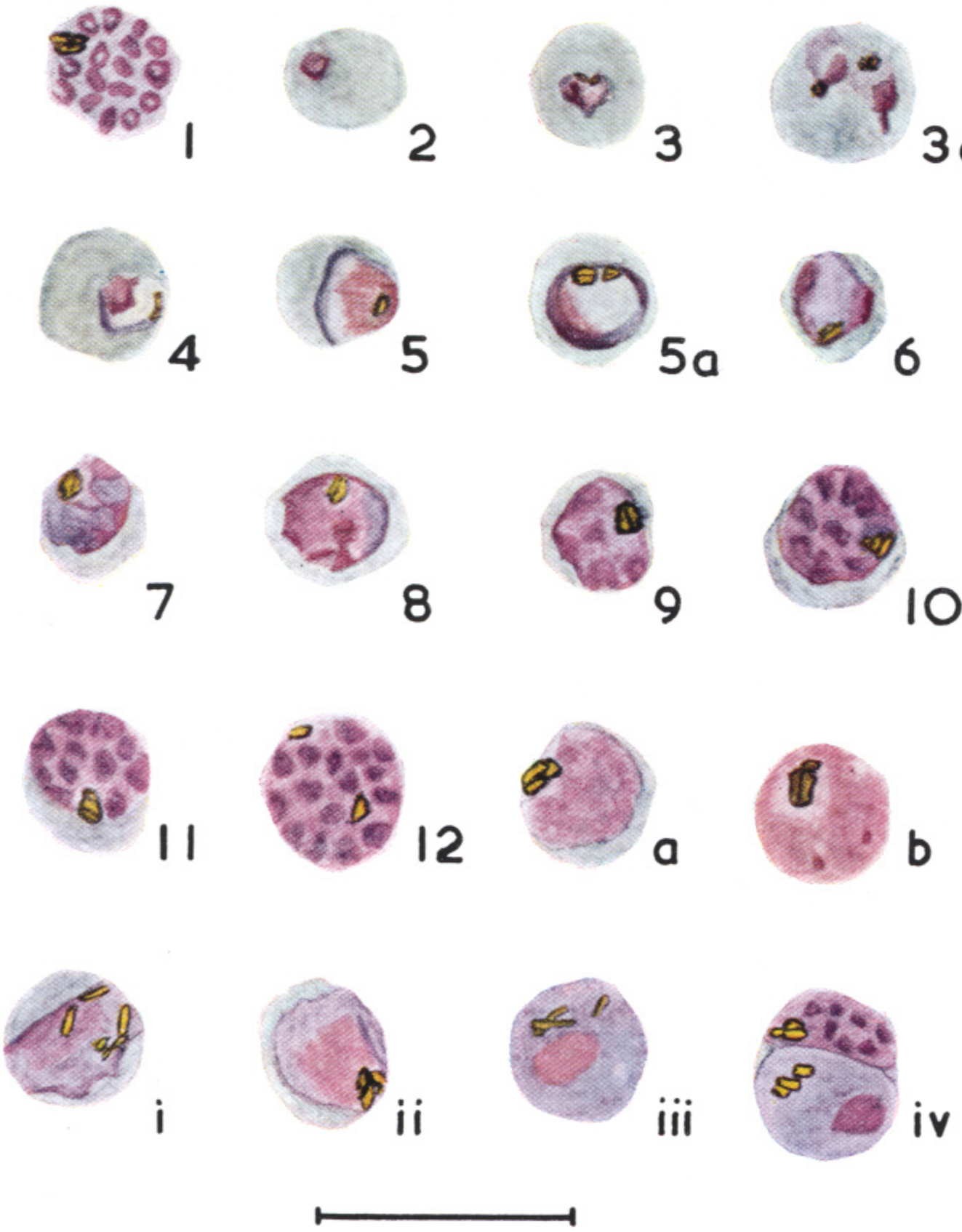
3

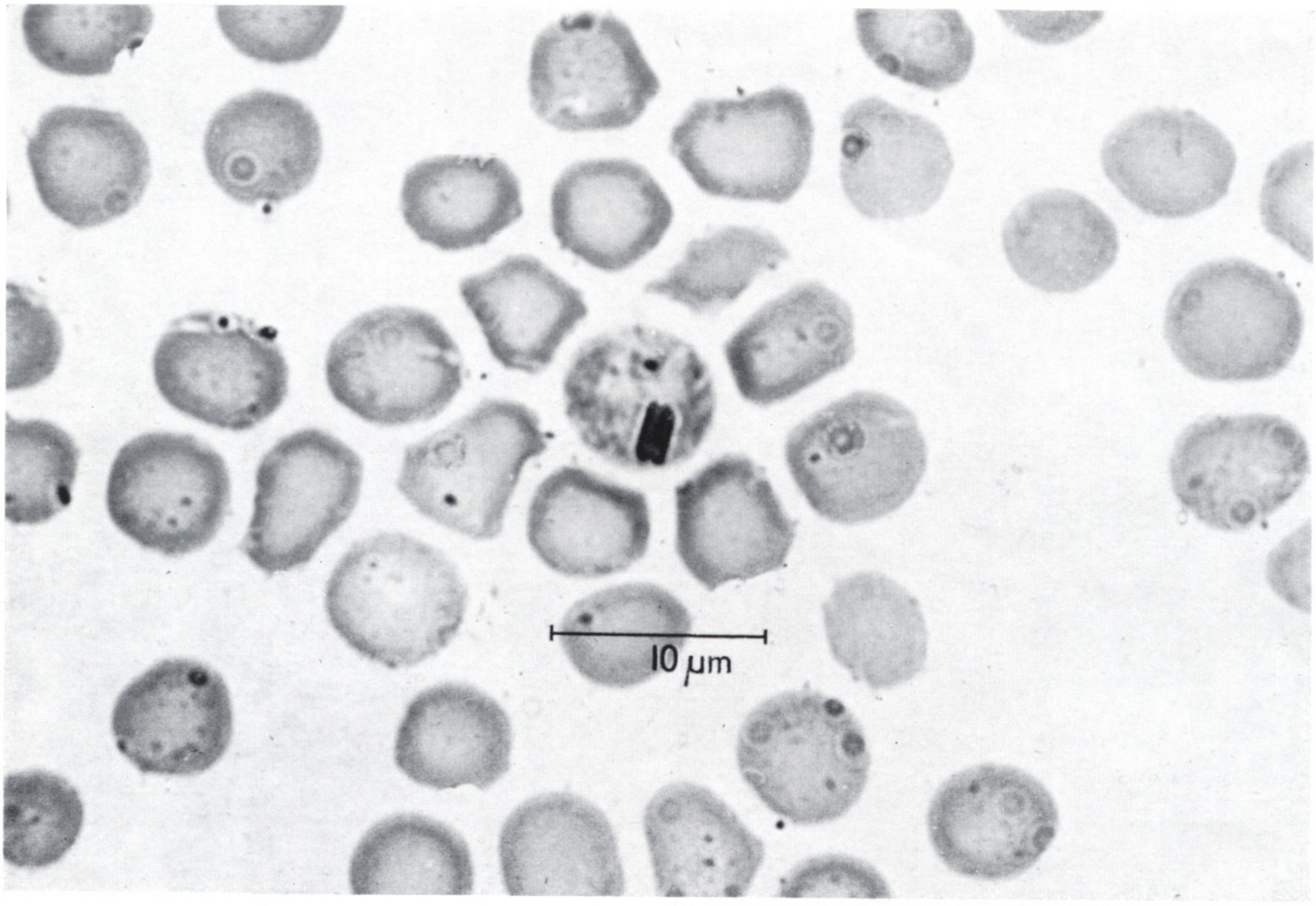
FIGURE 2. Special house for duikers in Habitat II*a*, Malawi.

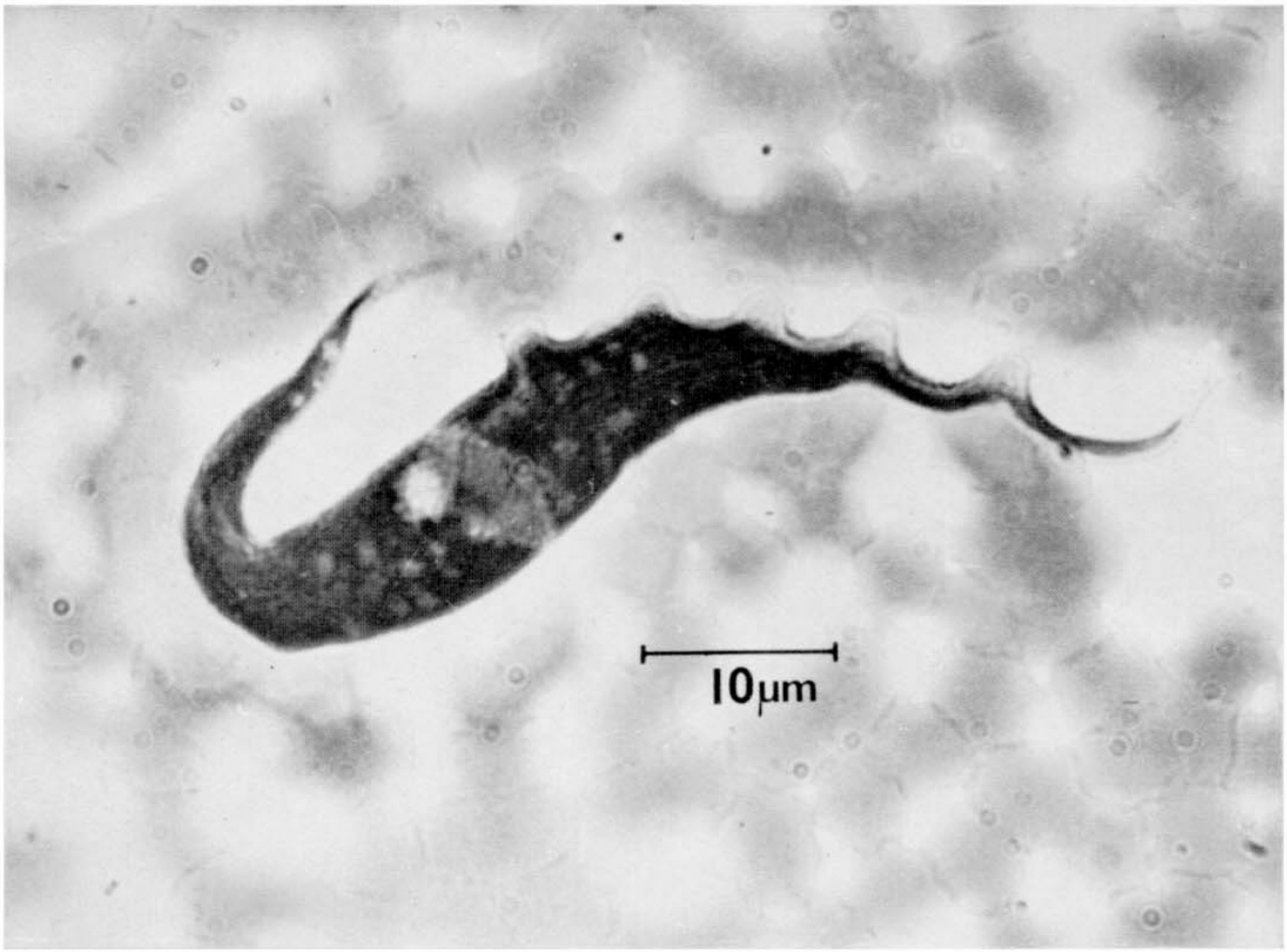
FIGURE 3. Adult male common duiker (*Sylvicapra grimmia*). It was in this splenectomized antelope that *Plasmodium* (*Vinckeia*) *cephalophi* was rediscovered.

FIGURE 4. Adult female Duiker (*Sylvicapra grimmia*).

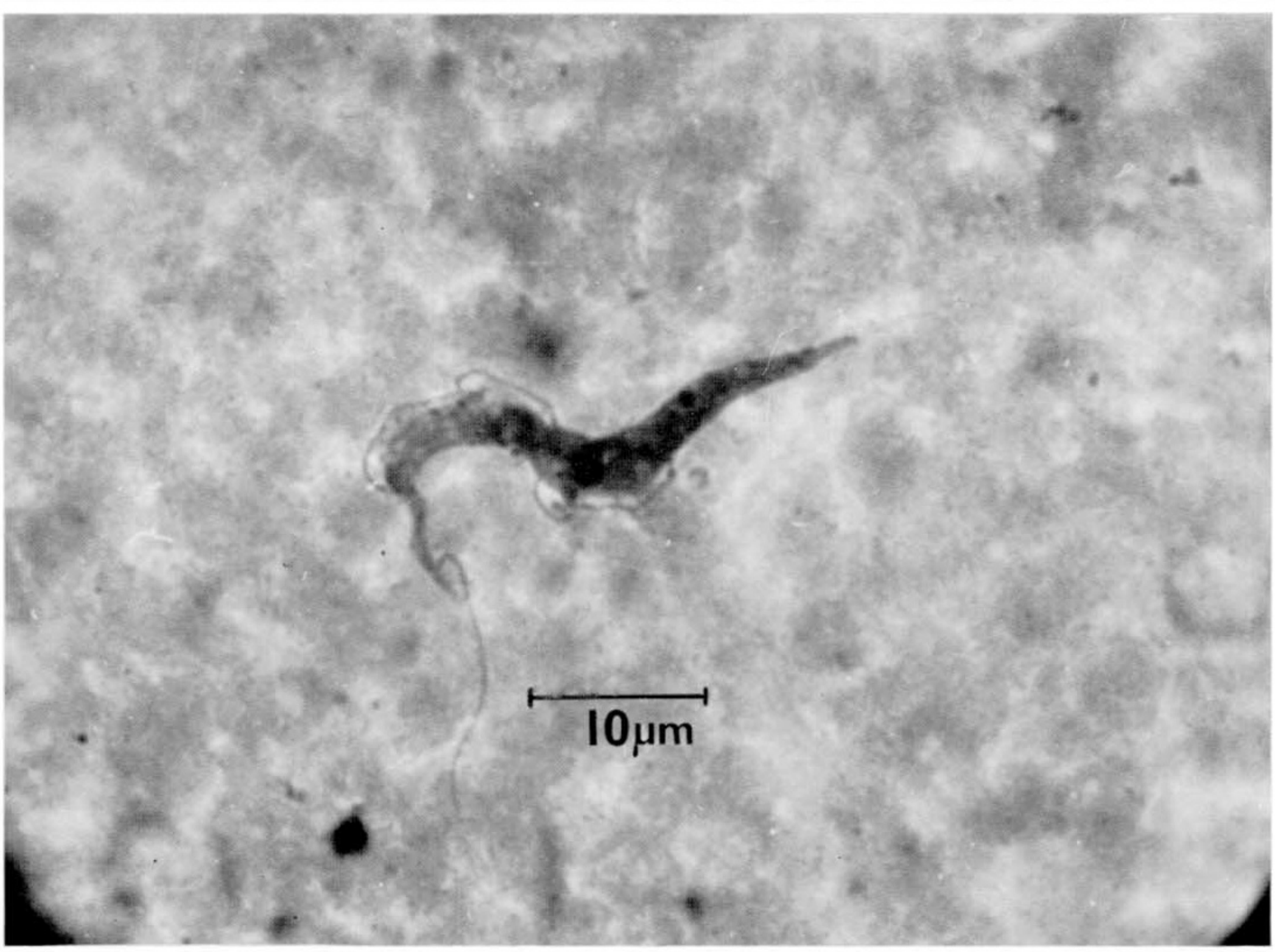
FIGURE 5. Baby duikers (*Sylvicapra grimmia*) with goat foster mother. After splenectomy, both of these antelopes became infected with *Plasmodium* (*Vinckeia*) *brucei*.


1
2
3
3a
4
5
5a
6
7
8
9
10
11
12
a
b
i
ii
iii
iv
10µm


10 µm



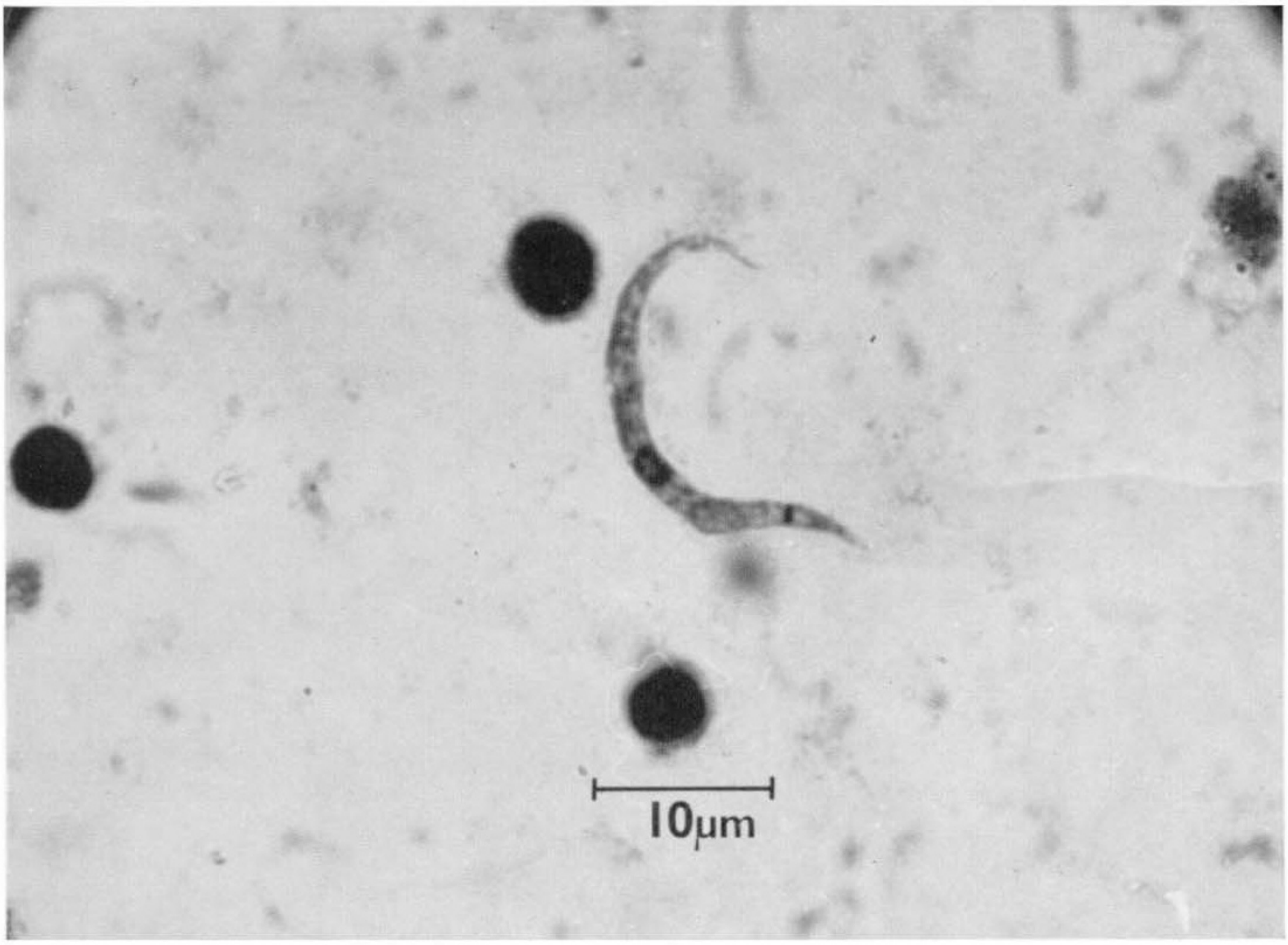
10



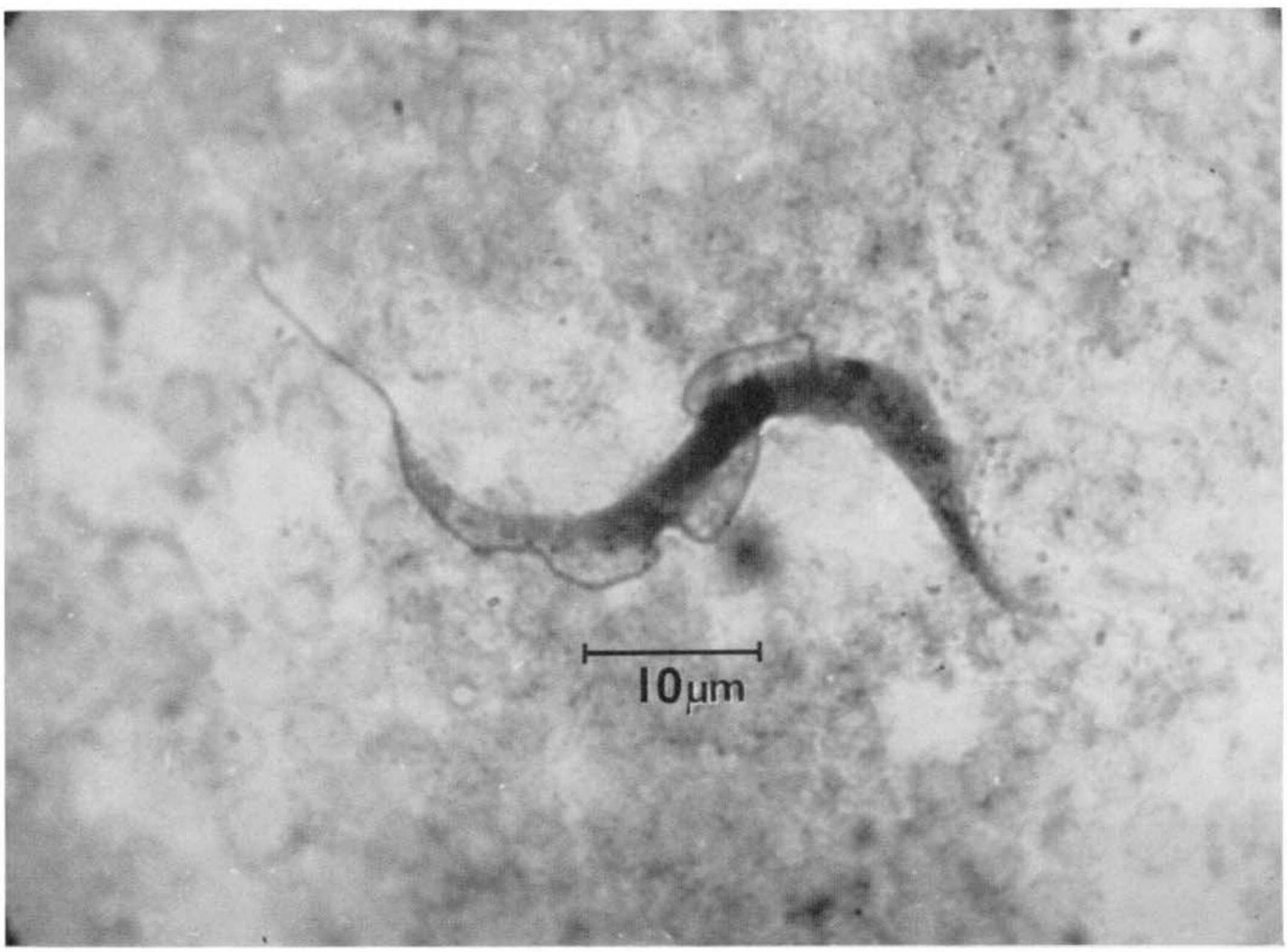
11

FIGURE 10. Photograph of a typical broad *theileri*-like trypanosome in a thin blood smear from duiker no. 226. Immediately posterior to the band-like nucleus there is a white vacuolated area. Adjacent to the latter is the round dark-staining kinetoplast. Longitudinal striations or 'myonemes' are visible both in the anterior and posterior parts of the body.

FIGURE 11. *Theileri*-like trypanosome in a thick blood smear from the duiker (*Sylvicapra grimmia*). Parasite from antelope no. 322 which showed a low level of parasitaemia. This organism corresponds to Theiler's 'ordinary form', the kinetoplast being situated at some distance from the nucleus, which in this parasite shows as a very dark staining oval area near the middle of the body.



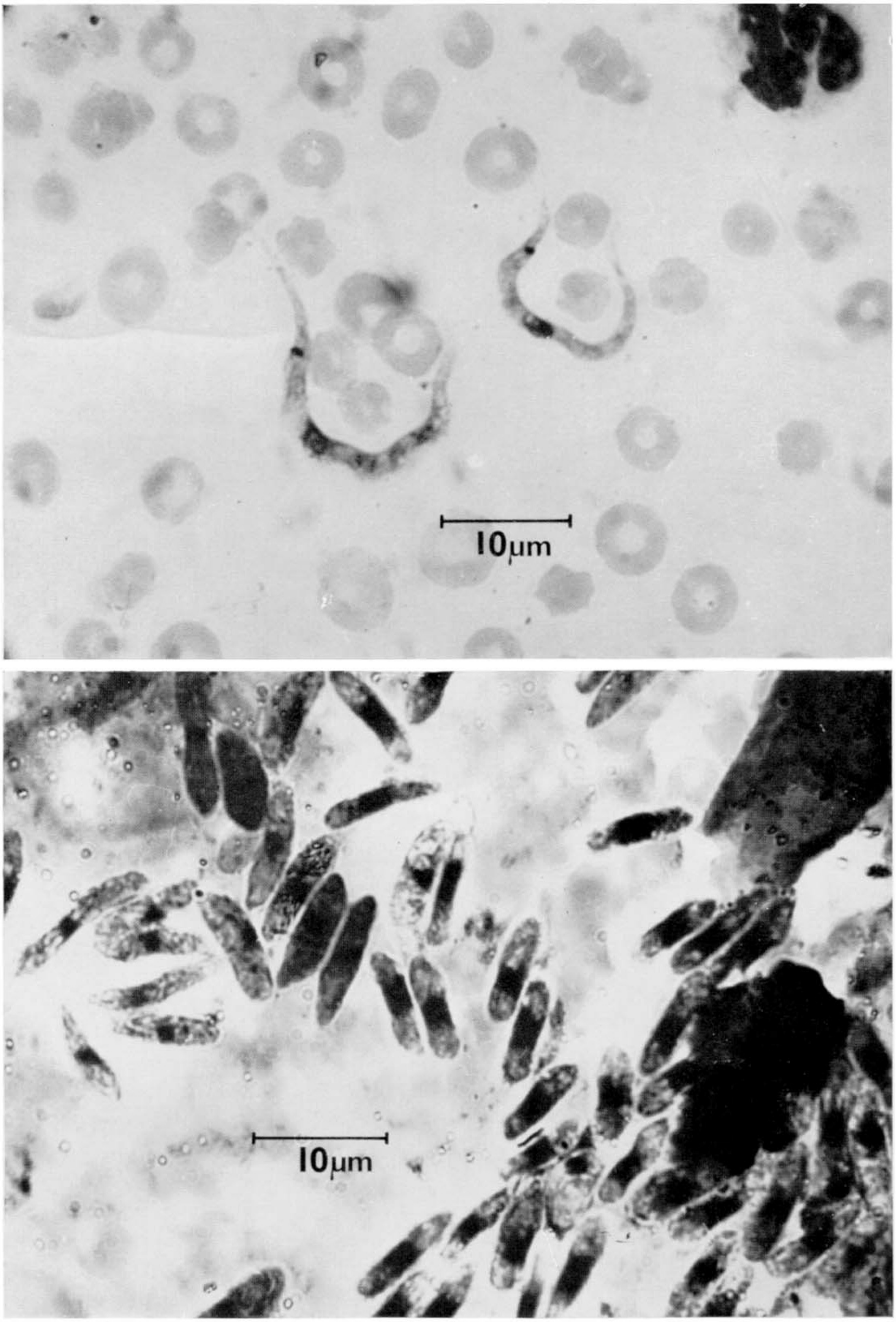
24



27

FIGURE 24. *Theileri*-like trypanosome; Theiler's 'ordinary form' with a *KI* value lower than 2, the kinetoplast being nearer the posterior end than the nucleus. Thick blood smear from Nigerian ox (*Bos taurus*).

FIGURE 27. *Theileri*-like trypanosome in a thick blood smear from a splenectomized calf (*Bos taurus*), 36th day after injection. This parasite corresponds to the *T. transvaaliense* type originally described by Laveran (1903). The kinetoplast is near the nucleus, and a considerable distance from the posterior end of the body.



28

32

FIGURE 28. Thin blood smear from Indian ox (*Bos taurus*). *Theileri*-like trypanosomes. The smaller parasite is typical of Theiler's 'ordinary form' with a *KI* value lower than 2. The larger parasite, however, having the kinetoplast rather nearer the nucleus than the posterior end, is intermediate in type between Theiler's 'ordinary form' and the '*T. transvaaliense*' type.

FIGURE 32. Photograph of *Sarcocystis* 'spores' as seen in a thin blood smear from a duiker (*Sylvicapra grimmia*), reference no. 226, from Malawi. The dark staining nucleus is visible in most 'spores', being situated near the centre of the parasite. The cytoplasm is pale staining and granular.