STUDIES ON ABODIA DIGITATA IN CATTLE IN NORTHERN SUDAN

A THESIS

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BY

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to

my mother and father who both died before the completion of this study and whose patience and personal sacrifices have done so much to me.
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تمحور أنواع البهاء من اسم لطيبات الدم النسي.

تيبة الأذاع في السواد، وبسه البهاء أو الرتبان،
أو حسن النزاع مخالى أنه ينتظى بالنزاع. ضحيلة النازل
الواضح، ولكن في السواد لم يتم أجراؤه، أي يحذى
على يرندة.

في عام 1379 هـ، وضع مجال البهاء في مدينة
(ج) على النزل الأقليق، ووضع على النزل الأبين.

وفي هذا البهاء تم أجراؤه، مصوح بعده للمؤسسات
التي تم فيه جميع معاهد من القواعد القليقة، الصنایع
والسماح بالمنفخ الصارم، وفقًا لعصاب الشكل.
(ه) في هذه الصناع فيما أجراء تم دورة
بمس القواعد في شتى المواقع.

وقد أُوجب تأكيج الشيء البهاء في روبر تغين من
البهاء، وهو من التربة، وذلك بالكشف والشمس، ووضع
النور في جبال النزاع، ثم أن القواعد من مادة النازل الواضح.

أقرب الرسالة على حضرموت، أجراء مصوح دليل
على الألوان الضخمة، كما ينبغي أن تكون تجربة نازل،
والنواب التي تصدح في البيئة، ونوع آخر يجب أن يكون له المرك
لتصلي البارون.
addition to two isolates; all were examined and studied in the laboratory at Sao. Experimental trials were carried out to determine the role of *Rhiphelus decoratus*, *Rhiphelus annulatus* and *Hyalomma anatolicum* in the transmission of *Babesia bigemina*.

The preliminary survey resulted in identification of *Babesia bovis*, *Babesia bigemina*, *Theileria annulata*, and *Theileria mutans* microscopically and serologically. The epizootiology of *Babesia bigemina* was unstable but the situation of tick-borne diseases in general is in need of detailed field and laboratory investigations of up to continuous 5 years. Experimental transmission trials resulted in considering the tick *Rhiphelus decoratus* as a natural efficient vector of *Babesia bigemina* in the northern Sudan. The role of this tick and other ticks in transmission of bovine babesiosis should be investigated intensively in the future.
CHAPTER 4

INTRODUCTION

1. General

Infection with Babesia parasites in animals derives its importance from its economical effects, causing substantial losses in production (Kistie 1951; Toderovic and Garcia 1978; Mackenzy, 1977). Recent work reported by many scientists, amongst whom were Kistie et al. (1971), Kistie (1961) and Healy et al. (1977), suggests the possibility of the emergence of Babesiosis as a zoonosis. As early as 1905 Balfour described a small piroplasm from the blood of cattle in the Sudan. Then Oliver 1907 found the organism of Biliary Fever (piroplasmosis) in korous and doss. Subsequently Babesial infections have been regularly reported from all over the country. So far, only two species of Babesia, B. kovia and B. bigeminum have been reported to occur in Sudanese cattle (Animal Wealth Administration annual reports 1950 to 1981 and FAO-Tick and Tick-borne Disease Control Project terminal report 1963, Sudan), the presence
"Large" Babesia. In small Babesia the pyriform bodies were between 1.0 um and 2.5 um and included B. bovis, B. divergens, B. suis, B. ovis, B. ribesi, B. domerguei, and B. rhodos. In the group of large Babesia, the pyriform bodies were between 2.5 um to 3.0 um and included B. bigemina, B. major, B. variabilis, B. malayi, B. tropis, and B. canis.

B. bigemina according to Bondy 1963 is a large pyriform, 4 um - 5 um in length and about 2 um width, round forms 2 um - 3 um in diameter. The organisms are characteristically pear-shaped and lie in pairs forming an acute angle in the erythrocyte. Round, oval or irregularly shaped forms may occur depending on the stage of development of the parasites in the red blood cell. B. bovis is a small Babesia measuring about 2.4 um by 1.5 um and slightly larger than B. divergens. Vaculated pigment ring forms are particularly common, consisting of a centrally placed vacuole with a nuclear mass at one end.
4. Babesial infections

4.1. Description of the Babesia

Babesia are intracytoplasmic parasites of vertebrates which are transmitted by Ixodid ticks. Mechanical transmission by biting flies however may occur (Barnell 1977). Infections with the babesial parasites are not always symptomatic, but the parasite or evidence of its presence can be detected in most animals in areas where the disease is endemic (Joyner and Donnelly, 1979).

In the past, species of Babesia have generally been regarded as strictly host-specific (Joyner and Donnelly, 1979), the concept of extreme host specificity may be challenged by new information. For example Gallow (1965) demonstrated that ticks carrying B. divergens may fail to become cleansed of the infection after feeding on non- bovine hosts which themselves may harbour the infection for a period. Significant change of the Babesia spp. host specificity concept
4.2.2. In ticks

Riek (1964) gave the first comprehensive description of the invertebrate phase of *B. bigemina*. After ingestion of infected blood by the vector tick, the early club-shaped body underwent multiple fission in the tick gut epithelium and resulted in a large number of vermicles which penetrated all tick tissues to continue the cycle. Initially, the vermicles had a homogeneous cytoplasm and a nucleus lying at the broader end to give a capped appearance. Vermicles migrated through the gut wall to the haemolymph, and as they aged the anterior red-staining area disappeared and the cytoplasm became vacuolated. By 72 hours onwards after repletion, such forms were present in the haemolymph, ovaries and other tissues of the body. Vermicles were initially in the yolk of the egg, then they enter the gut epithelium cells of the larva. Further development produced vermicles similar to those occurring in the major tick. These vermicles were then liberated into the gut lumen or haemolymph of the larva, (Riek 1964).
Development of *B. microplus* to the infective stage to the bovine host is dependent on a molt from larval to nymphal stage of the tick *Boophilus microplus* after which vermilocules become present in the cells of salivary glands and assume a spherical form, they enlarge and their chromatin scattered and broken up into large number of small particles. The cytoplasm then become organized around the chromatin particles to produce spherical or pyriform organisms which Rook 1964 regarded as mature infective forms ready for liberation from the salivary glands.

4.2.2. In the vertebrate host

Haynes (1961, 1965) reported that, after the tick had inoculated *Babesia*, it directly passed to the blood stream and infected the erythrocytes. The entry process of *Babesia* into erythrocytes was known until 1961, when Ward and Jack found that the process occurred in five steps.

5.1. Clinical recognition, the examination of smears and pathology of *Babesia*

Maloney (1977) stated that whenever a disease
clinically resembling babesiosis is observed, the organism must be specifically identified since there are a number of other diseases with which the clinical signs may be confused. Microscopic examination of smears is, therefore, the cornerstone of diagnostic procedures for the acute disease.

Many of the pathologic effects are due to the effects of parasitale multiplication in the host (Ristic, 1970). Anaemia is caused by escaping babesia as reported by Calico and Pepper (1974). Ristic (1970) concluded that fever, anaemia and parasitemia were considered to be valid parameters of the intensity of host—parasite interaction. Some Babesia species such as B. bovis and B. canis cause clogging of small capillaries of skin and brain (Ristic, 1970).

Ranefin (1977) studied the clinical signs liver lesions and kidney lesions of B. hydysycri in mice and observed symptoms and lesions similar

Fujinaka and Mizui (1961) indicated the effectiveness of the IFNa test.
CHAPTER II

MATERIALS AND METHODS

1. Field investigation
   1.1. Study area
   1.1.1. Description of the area

   An outbreak of leishmaniasis occurred in 1979 at Sagadi and was reported by the vector biology department of Animal Health and by FAO Tick Control Project, Sanaa. Sagadi area (11° 30' N 39° 0' E) is a cluster of four settlements around the foot of Sagadi mountains (jebel). The area is about 350 km south of Khartoum and 65 km north west of Sennar town.

   It lies at the southern boundary of the irrigated area where cotton and crops are cultivated. The people are mostly farmers growing various crops in the rainy seasons in the poor surrounding ground
The natural grazing areas in this region are adversely affected by the continuously increasing traditional rain-fed and irrigated farming systems. During the rainy season to the south of the area the ground becomes waterlogged and great numbers of biting flies attack the animals so that animals owners move northward and Sagadi area is one of their chosen places.

1.1.2. Climate

Meteorological data for Semmar town and Koobi town (13° 28’N 33° 30’E and 13° 10’N 32° 43’E, respectively) recorded for a long period (30 years), can apply for Sagadi. Between June and September average rainfall at Semmar was 463 mm and at Koobi 404 mm. Mean daily air temperature ranged from 14.9 °C minimum in January to 31.1 °C maximum in April and May. Mean relative humidity ranged from 21 in April to 69 in September.
Photograph 1

A photograph from Sagadi Jebel showing one village of Sagadi, (Site of collection)

1. Water-point, Hafir
2. People houses and cattle enclosures
3. Sagadi Jebel or Mountain

Photograph 2

Sorghum sticks on which cattle are fed. This naturally after harvesting of Sorghum, other seasons cattle are grazing on very poor natural pasture surrounding Sagadi.
1.2. Live-stock Husbandry

Cattle in the area are mainly Kemenana short-horned Zebu type. Cattle at Sagadi are enclosed in thorn-bush enclosures (Zerebas) during night. Calves are enclosed separately. All animals including cattle graze the area around the Sagadi. Management as practiced by these semi-nomads is traditionally poor. Water is either drawn by hand from wells or pumped from "hafire" (dog-out excavated in low-lying areas), which becomes filled with rain and may last till the next rains. There were five hafires and three wells at Sagadi. Cattle are watered once a day.

1.3. Survey and examination of animals

The survey was done only during the seven months from January to July; it was not possible to survey during the rainy season (July to October). The four
Sagadi middle (SM), Sagadi west (SW), Sagadi east (SE) and Sagadi north (SN). Sagadi middle due to its cooperative people was studied more intensively and most of samples were from it. So efficient and easy sampling and follow-up of cattle was obtained.

Daily visits were made before animals walk out to pasture. During these visits owners brought their sick animals for treatment. Such animals were clinically examined and samples of the possible disease, blood for serum, blood film and total body ticks collection were taken. Samples also were taken from other animals at random.

Records of the cattle examined were kept containing serial numbers, the owner name, disease suspected, age of animal, sex and breed, tentative
diagnosis, samples collected and treatment, if any, given. Animals of each of the four villages were recorded separately and each animal species was separately recorded also.

1.4. Collection of samples from the field

1.4.1. Blood smear

From cattle presented for clinical examination and other cattle randomly selected peripheral blood smears were collected regularly. A drop of blood from ear or tail vein was obtained in clean microscopic slide. Preparation of blood smears was done according to McCooker 1975, (see 1.5.). Blood films of suspected cases of babesiosis after clinical examination were fixed with methyl alcohol air dried and then stained with Giemsa. Sunlight microscope (x100) was used for the suspected babesiosis blood film at Sagadi. Blood slides were labelled as follows; village name e.g. 3m; no. of the case e.g. 3m 101 and the date. This was done with pencil.
before fixation on the smear or by diamond pencil on the slide glass.

1.4.2. Serum and blood spots

Serum was collected from restrained cattle through jugular puncture. Blood was collected in serum vacutainer and left in ice-box containing ice overnight. Next day the ice-box with its contents were transferred to Bennar town laboratory (65 km) where the oles removed and the serum centrifuged and was obtained in Bijou bottles, labelled, kept in freezer for few days before being sent to John laboratory, Khartoum Province.

Most of cattle owners were reluctant for serum collection so, blood spots were collected according to Burridge et al. (1972). Bar vein puncture was made, whatman filter paper No. 4 was attached to the o责令 blood until the blood covered a circular area of at least 1.5 cm in diameter. The filter paper blood was allowed to dry, labelled with the
animal case no., village and date of collection. Each
25 filter papers were wrapped in plastic bag and put
in ice-box and sent to Soba laboratory.

1.4.3. Collection of infected blood

From cattle found microscopically positive for
Babesia spp., 40 ml whole blood in EDTA vacutainers
was collected. The case label comprised of owner
name, cluster or village name, breed of cattle, age
and sex, parasitemia, time and date of collection.
The vacutainers were then immediately transferred in
ice to Soba laboratory and immediately on arrival
blood stabilate was made.

1.4.4. Ticks collection

Collections of ticks from whole body of most of
the examined animals were made with tick forceps.
Larvae, nymphs and partially emerged ticks from each
animal were collected in one universal bottle
containing 70% methyl alcohol as a preservative.
Semi-engorged and engorged ticks were collected in clean empty tick tubes and transferred to the laboratory in humid jars. Each tube was labelled with the animal case number, village name and date, practically the same label of blood smear and serum. Labelling was done in this manner to correlate the blood film result with tick infectivity test especially for Boophilus spp.

1.5. Field isolation
1.5.1. First isolation

A one-year-old, Kembrana calf was presented as a sick calf. Clinical examination of the calf showed temperature 40.5°C, preocular and car lymph nodes enlargement, pneumatic lungs and the hind legs scoured with diarrhea and tropical theileriosis was suspected as a possible disease. Blood smears were then made from ear vein, fixed with methanol and stained with Giemsa for microscopical examination. Theileria spp., Babesia spp., and Anaplasma spp. were all seen in the
blood films. Infected blood was immediately collected (see 1.4.3.) and transferred to Sahn. Stabilate no. 51 was made.

1.5.2. Second isolation

Two calves were presented sick for treatment. The first one S\# 15 was 1½ year old Kamana calf, almost recumbent and lachrymating. Rectal temperature was 41.5 °C and ear lymph nodes were enlarged. The second was S\# 16 Kamana calf 2 year old was presented for treatment for mange. Rectal temperature was 40.5 °C, prescapular lymph nodes were enlarged.

On microscopical examinations of their blood films Babesia spp. and Theileria spp. were seen in both. Blood in EDTA was collected from both calves and transferred to laboratory where stabilate 52 was prepared from both cases.

1.5.3. Infectivity tests

Stabilate 51 when inoculated in clean splen-
centimized calf no. A 54 no parasites was seen in the calf peripheral blood for 60 days post- inoculation and no fever response was recorded. No antibodies were detected in all weekly collected sera against *E. mutans, E. annulata, B. higromic* and *B. bovis* stabilis 52 was inoculated into calf A 37 resulted in successful transmission (see chapter IV).

2. **Laboratory investigations**
   2.1. **Experimental Animals**
      2.1.1. **Calves**
         2.1.1.1. **Source of clean calves**
           
           The calves were highgrade crossbred calves (Bos taurus X Bos indicus), obtained from Belgrade dairy where routine acaricidal control of ticks is practiced. No report of disease due to *Rickettsia* species experienced during this study in the farm.

      2.1.1.2. **Rearing of calves**
           
           Calfes were reared in tick-proof pens. Each
2.1.3. Feeding of calves

The calves were obtained at 1-3 days old and were fed on freshly boiled cow milk. Each receiving 9 pints daily during the first month and 6 pints and 3 pints during the second and third month of age respectively. At two months old the calves were introduced to concentrate feeding. They were fed on manufactured pellets to avoid tick infestation hazards if fed on green fodder or Sarsoam.

2.2.1.4. Acaricidal spraying

As a further precaution against accidental tick transmission, the rooms, the pens and the calves, were regularly sprayed with short residual acaricide Vasona Smell (R) at a concentration of 1 : 100 in water.
The tick-proof pens were built in two adjacent rooms and separated by a solid wall. Calves on tick feeding experiments were kept in one room and those on regular spraying in the other room.

2.1.1.5. Calves susceptibility

Calves were subjected to daily routine of temperature recording and peripheral blood smear examination from day of arrival till end of the experiment or the animal death. Serum was taken on arrival of calves and then weekly or as the experiment required and tested for Babesia spp. antibodies by the indirect fluorescent antibody technique (IFA).

Calves were considered clean and susceptible if they showed normal temperature and no parasitic parasite seen in the blood films nor antibodies detected against Babesia spp. in the sera. This procedure was followed for at least one month before
experimental transmission were started.

2.1.6. **Appendectomy of calves**

Calves were starved for 24 hours to reduce the rumen contents. The left flank was clipped and disinfected immediately before surgery. Lidocaine hydrochloride anhydrous, 20 mg/ml (Lidocaine 2%) was used as local infiltration anaesthesia on the operation site. The tranquiliser, Xylazine 20 mg/ml (regnun vet) was injected intramuscularly at dose rate equal 0.1 ml/ 10 kg body weight following the local anaesthesia application.

The operation was started with the calf lying on its right side. The incision was made in the left paralumbar fossae, beginning antero-dorsally as far as possible into the angle formed by the last rib and the transverse processes and continuing close to and parallel with the last rib for about 12 cm. All connective tissue attachments of the spleen to the rumen, reticulum and left crus of the
diaphragm was broken down with fingers. The splenic vessels and nerves were found at the hilus by pressing the hand over the parietal surface and palpating the arterial pulse a short distance medial to the anterior border. The splenic vessels and nerves were clamped with artery forceps and firmly ligated with 2-0 P. plain cat-gut suture. Two ligatures were made one near the anterior extremity and the other about 1" anterior to it. The splenic stump was cut off between the two ligatures. After removal of the spleen the peritoneum, transverse abdominalis and internal abdominalis, and external abdominalis muscles were sutured separately by simple continuous suture using 2-0 P. plain cat-gut suture. The skin was closed by interrupted suture using monofilament nylon. B.P.C. which was removed 10 days later. After the operation the calf was given 1/10 injection of 7 ml of streptomycin (procaine penicillin 250,000 units/dihydrostreptomycin) prophylactically.
2.1.2.7. Calves routine observations

All experimental calves before and during experiments were daily examined clinically. Daily peripheral blood (ears or tail veins) smears were taken, stained with Giemsa and examined.

2.1.2. Rabbits

Rabbits were purchased from Khartoum Public Market, kept in metal, easily washed cages. All rabbits cages were placed in tick-proof pens and sprayed regularly or according to the experiments programmes. Rabbits were clinically examined daily. They were fed on millets.

2.2. Infection of calves

2.2.1. Blood stabilates inoculation

A known blood stabilates of known parasitaemia was inoculated in clean splenectomised calf. The calf subjected to daily routine observations as discussed
Before and to daily blood film examination.

2.2.2. Infestation via ticks

2.2.2.1. Calves preparation

Calves on experimental transmission programme were kept in one room i.e. not under regular vapour spraying programmes (see 1.1.4.). To remove any acaricide residual the calf's whole body was washed with water and soap for two consecutive days, then left for one day to dry. The ears in addition to washing with soap and water were cleaned of wax and secretions with cotton wool moistened in 70% methyl or ethyl alcohol solution.

2.2.2.2. Ticks applications

For Boophilus spp. ticks whole body feeding was allowed. A leather belt of 20 cm wide and 100 length was used for application. On the belt surface which was attached to the animal skin 10 cloth pockets were
prepared, each pocket accommodated one plastic tick tube measuring 3" x 1". The Boophilus annulatus larvae tubes were firstly placed in the pockets; not more than 10 tubes were used in each one application, then the belt with the open ends of the tubes towards the animal skin was fastened around the calf's chest and back. This method gave the larvae ample time to be released gently and get attached to the calf body.

For Hyalomma anatolicum anatolicum larvae, nymphs and adults an open-ended ear-bag made of cotton cloth was fixed to the ear with Zinc Oxide tape (Paragon tape) and it was found very efficient according to Al Rana (1983). The tick tubes were introduced into the ear-bag by the free end which was then closed with elastic band. The tube cover was removed to let the ticks free to get attached to the ear. Empty tubes and dead ticks were removed the next day. Engorged ticks were collected by shaking the ear in plastic bag. Each calf was isolated in a tick-proof pen with restrained hind legs to
2.3. The Ticks

2.3.1. Source of ticks

Boophilus annulatus ticks were obtained from Sagra, They were removed off cattle as semi-engorged females. One batch of Boophilus annulatus Kadugli strain (13° 30'N 29° 35'E in south west Sudan) was kindly sent by Dr. M.A. Omer. *Haemaphysalis anatolica* (Kastoria strain) were obtained from Shambat and Bagair (Khartoum Province 15° 40'N 32° 35'E). Ticks were transferred to the laboratory at Sola (Khartoum Province) in plastic tick tubes covered with nylon mesh secured by an elastic band or plugged by cotton wool wrapped with gauze. Tubes were put in Kilner jars and placed in ice-box containing ice. Adults were immediately identified using a microscope (Olympus) according to Hoogstraal (1956) and Hoogstraal and Kaisen (1959). Larvae and nymphs were identified
after molting to reach the adult stage accordingly.

2.3.2. Breeding of ticks in the laboratory

Engorged females were put singly in 3" x 1" glass tubes containing a piece of filter paper Whatman No. 4. Eggs and immature larvae and nymphs were separated in 3" x 3/4" glass tubes. All tubes were put in Killner jars with cotton wool moistened with saturated sodium chloride (NaCl) to provide 75% RH (Relative humidity). For *Boophilus* spp., cotton wool moistened with tap water to provide 100% RH and drops of diluted mycostatin solution were added to the moistened cotton to prevent growth of fungi. Every jar contained one *B. capaxis* tick. Jars were closed firmly and put in a low temperature incubator (Astel, Pearson England). Eggs and engorged ticks were incubated at 28 °C while flat ticks at 20 °C.

2.3.3. Feeding of ticks

2.3.3.1. On calves
2.1.32. **On bubblis**

Bar-bag feedings were carried out in a similar way to that described for calves. Rabbit masts were shortened with scissors to prevent them from tearing and removing the ear bags.

2.1.4. **Tick Handling and labelling**

Emerged Boophilus spp. females were collected from the infested calf pen floor 3 times daily, in the morning, after noon and at sunset. Collected ticks were put singly in glass tubes and each tick had a serial number starting from one. Eggs were left in the same tubes after removal of dead ones and in some instances they were divided by water-colour brush into small tubes. *R. microplus, R. microplus* larvae and nymphs were counted during the inactive stages. Equal numbers of female and male were applied to animals during feeding trials of adults.
2.4.2. Giemsa staining

GUNN improved Röö Giemsa stain solution (Hopkin and Williams, England) was used. The Giemsa solution was diluted in buffered distilled water (BDW) at pH 7.2 and used immediately for staining. One part of Giemsa to nine parts of BDW was used and the air-dried fixed smears were immersed in the solution for half an hour and then washed with BDW and air-dried. For each batch of fixed slides new Giemsa solution was prepared from the stock solution. The BDW was prepared by adding one pH 7.2 buffer tablet (Hopkin and Williams, England) to one litre of distilled water.

2.4.3. Smear examination

The smears were examined under Leitz (diavox 20) microscope using x 100 immersion objective. At least 3000 red blood cells (rbc's) were counted and Babesia spp. parasitaemia was expressed as average number of cells infected/1000 red blood cells (rbc's).
2.5. Collection of serum

Experimental calves were bled for serum collection on the first day of arrival, before splenectomy and thereafter weekly during and after an experiment. Blood was collected in sterile empty evacuated vacutainer by jugular vein puncture and kept at 4 °C overnight. Then the clot was removed and the serum centrifuged at 2000 r.p.m. for 10 minutes on MSE minor centrifuge. The serum then divided into 2 small vials and kept at 20 °C.

2.6. Blood stabilise preparation

Ismadhan standard technique for blood stabilise preparation (1978) was used. Blood for stabilise preparation either received from Jagadi age 1.4+2 or from known nonasmatoid experimental calf. Either glycerol or dimethyl sulphoxide (DMSO) at concentration of 10% was used as a cryoprotectant. The mixture of blood and the cryoprotectant was equilibrated by gentle shaking for half an hour at room temperature
before being distributed into cryopreservation plastic vials in 5 ml and 2 ml aliquots. The vials were labelled with stimlate number and then were frozen down by slow cooling (approximate rate of 1 °C/minute). This was achieved by placing the vials in 2 inch thick polystyrene insulated box in Revco (-70 °C) for at least 24 hours and then transferring the vials directly to liquid nitrogen.

Stabilate records were kept in a special stabilate file. These records included a special number for each stabilate, parasite, strain, original host, total number of stabilate aliquots, canister number in the liquid nitrogen container and results of infectivity test.

Dalgliesh (1971) reported that (OMSG) caused a slight loss of infectivity before freezing. Subsequently inoculated frozen blood with glycerol was highly infective (Dalgliesh 1972) but intravenous inoculation of glycerolized blood caused marked loss of infectivity.
3. The indirect fluorescent antibody test (IFAT)

3.1. Serum collection

Serum collected from Sagadi (see 1.4.2.) and five experimental calves (see 2.5.).

3.2. Antigens preparation and control sera

*R. bigemina* antigen was prepared from blood of experimental calf A56 inoculated with Sagadi strain *R. bigemina*. Another more specific antigen was prepared from calf A61 which was tick infected. 10 ml blood was directly transferred into about 200 ml phosphate buffer saline (PBS) and then centrifuged at 1000 r.p.m. for 10 minutes, the supernatant fluid removed and the process was repeated 4 times. The precipitate was spread on precoated microscopical glass slides. The smear covered the whole slide, air dried and then fixed in fresh acetone for 10 minutes. Suitable smears were chosen and groups of five were wrapped in tissue paper and then with tinfoil. Each group was labelled with the antigen
number, dated and then transferred to the Revvo
(-70 °C) for storage.

*E. bovis* infected blood originally a Nigerian
strain was obtained from Prof. Ulbergh. *T. carsoni*
mutans antigen was from a Sagadi strain and
*T. annulate* antigen from a Soba strain. Preparation
of these three antigens was practically the same as
*E. bigenina*. *E. bigenina* and *T. annulate* positive
controls with a titre of 1/2560 were obtained from
Centre for Tropical Veterinary Medicine, Edinburgh.
*T. mutans* with titre of 1/100 was obtained from Kenya
Agricultural Research Institute, Masaga, Kenya.

*E. bovis* positive control was obtained from Prof.
Ulbergh. The negative control was obtained from
Centre for Tropical Veterinary Medicine Edinburgh.
The conjugate (rabbit anti bovine gamma globulin
conjugated with fluorescein isothiocyanate) was
purchased from Nordic Immunological Laboratory,
Netherlands.
3.3. The (IFA) test

3.3.1. Sera testing

The IFA test was done according to the method of Burridge (1971). A group of 40 sera was used for one day test. Frozen sera thawed at room temperature and 0.05 ml from each serum was diluted in phosphate buffered saline (PBS) at 1/30. A group of five slides of each antigen were transferred gradually from -70 °C till room temperature. Each 8 sera were applied in 8 circles on the antigen slide and the negative and positive controls in two circles, slides incubated at 36 °C for 1/2 hour. Then all slides washed with (PBS) and air dried. The conjugate then was applied in all circles and incubated for 1/2 hour and then washed and air dried. There was a special paper for each test for result reading.

3.3.2. Dried blood spots testing

This was done according to the method of Burridge et al. (1974). 5 discs of 5 mm diameter
of dried blood were eluted in 0.5 ml PBS for 2 hours at room temperature. It was determined that a dilution of approximately 1/100 was obtained. The elution was the source of antibodies and the test then was performed as for sera.

1.4. The (IFA) test reading

Olympus fluorescent microscope was used for the reading. The light source was a J.C. super high pressure mercury lamp type USH-2000M manufactured by USH10 Electric Co. Japan, the Barrier filter was No. 0515 and the exciter filter was No 3.
CHAPTER III

QUANTITATIVE EPIDEMIOLOGICAL SURVEY ON PREVALENCE OF TICKS AND ENDOCUTANEOUS TICK-BORNE DISEASES WITH SPECIAL REFERENCE TO BOVINE BUBONISIASIS, AT SAGADI

1. INTRODUCTION

An outbreak of babesiosis occurred in 1979 at Sagadi 13° 30'N 33° 0'E, and reported by the FAO-Tick and Tick-borne Diseases Control Project, Sudan 1983, and Epizootic Control Department of Animal Wealth Administration, 1979. *B. bovis* and *B. bigemina* were diagnosed to be the cause of the outbreak, but neither quantitative studies on bovine babesiosis nor intensive laboratory investigations were carried.

In Sudan *B. bigemina* was regularly reported clinically and eventually confirmed microscopically (Karib Personal Communication, and Animal Wealth Administration Reports 1962 - 1927 and 1960 - 1962).
Bol-Dam, Yarakan and Abu-Sifeir are some of the native names given to bovine babesiosis in Sudan (Shommein Personal Communication 1983). With the exception of the reported outbreak at Sagadi 1979 which caused high mortalities, the disease although widely spread in Sudan is of low mortalities in the local cattle.

Hoogstraal (1966) couldn’t find the tick _A. a._ spartaculum at latitude 19º30'N and latitude 25ºN was about the northern limit of the tick _Boophilus annulatus_ the possible vector of _B. bovis_ (Nazariea personal communication 1992). The tick _Boophilus microplus_ was not recognized in Sudan by Hoogstraal in 1966 but he stated that there was some likelihood that it might reach Sudan within a few years.

2. Babesiosis at Sagadi

2.1. Clinical examination of cattle

A total of 738 head of cattle were examined clinically. Total number of cattle counted at
Sagadi during the survey period (from January to July) was about 1500 head. All the cattle owners not engaged in farming usually travel with their cattle southward searching for more food and water and come back at the beginning of rain fall.

2.2. Clinical examination result

Only 5 heads out of the 738 showed symptoms of Yarakin or Bol-dam (Yellowish mucous membranes or bloody urine).

Two cases died due to babesiosis. Post-mortem lesions of the two carcasses were diffuse jaundice in all subcutaneous muscles and the mucous membranes. Very thick bile with enlarged, fragile liver was noticed in both carcasses. The spleens were enlarged at least twice their normal size. The lung of one carcass was pneumatic and the heart flabby. The kidneys of both were inflated and slightly enlarged and mottled.
Table 1.

Clinical babesiosis at Saradi during the survey

<table>
<thead>
<tr>
<th>Total No. examined</th>
<th>Cases of Babesiosis</th>
<th>Mortality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>736</td>
<td>5 (0.68)</td>
<td>2 (0.27)</td>
</tr>
</tbody>
</table>

Figures in parentheses are % of total number sampled 736.
3. Microscopical examination of blood films

Peripheral blood smears were taken from all the 738 cattle examined. Some of these smears were examined at Sagadi camp with sunlight microscope but these were subjected again for further laboratory microscope examination (Leitz, dialux 20).

Blood parasites detectability in Sagadi survey can be summarised as follows:

a. *E. bighorn*, 35 head of cattle (4.74%)

b. *E. bovis* was found mixed with *E. bighorn* in 3 occasions (0.44%).

c. *Theileria species* (*Theileria mitans* and *Theileria annulata*) were detected in 135 cases (18.29%). Another 15 cases (2.03%) of *T. spp.* were found to be mixed with *E. bighorn* and 8 slides (1.05%) were found to be mixed with *Anaplasma spp.*

d. *Anaplasma spp.* was found unmixed in 3 animals (0.40%).
Table 2.

E. histolytica, blood parasites, and E. bigemina on blood smears

<table>
<thead>
<tr>
<th>Total No.</th>
<th>nps</th>
<th>Blood parasites</th>
<th>E. bigemina</th>
</tr>
</thead>
<tbody>
<tr>
<td>738</td>
<td>539 (73.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>191 (26.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 (4.74)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

nps: no parasites seen
and mixed infections.

( ) Figures in parentheses are percentage of total number sample.
4. **Indirect Fluorescent Antibody Test**

4.1. **Cattle sera**

The cattle total number of sera used were 176. The serum, the conjugate, the positive and negative controls were all diluted in phosphate buffered saline (PBS) at 1/50 dilution. Antigens used were *B. bigemina*, *B. bovis*, *I. persulcatus* and *I. annulata*.

4.2. **Dried whole blood spots**

125 whole blood spots were collected and tested for presence of antibodies against the 4 previously mentioned parasites. The dilutions were the same as used for sera except that for dried spots approximately 1/100 dilution was used.

5. **Ticks prevalent at Sawadi**

Total body collection of ticks was done from 160 head of restrained cattle. Collected ticks were preserved in 10% formalin or 70% alcohol (Methanol).
Table 2.

Results of IFA test of Sagadi sera

<table>
<thead>
<tr>
<th>Antigen</th>
<th>IFA test result (sera)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>strong positive</td>
</tr>
<tr>
<td><em>B. bigemina</em></td>
<td>81(45.5)</td>
</tr>
<tr>
<td><em>E. bovis</em></td>
<td>30(16.3)</td>
</tr>
<tr>
<td><em>T. annulata</em></td>
<td>42(23.0)</td>
</tr>
<tr>
<td><em>G. mutans</em></td>
<td>138(77.5)</td>
</tr>
</tbody>
</table>

Total no. in sample 174

Figures in parentheses are % of total number in sample.
### Table 4.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>IPA test (Dried blood spots)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>strong positive</td>
</tr>
<tr>
<td>E. b. bigemina</td>
<td>64(51.2)</td>
</tr>
<tr>
<td>E. h. bovis</td>
<td>10(8.0)</td>
</tr>
<tr>
<td>T. annulata</td>
<td>13(15.2)</td>
</tr>
<tr>
<td>T. mutans</td>
<td>105(84.0)</td>
</tr>
</tbody>
</table>

Total number in sample 125

Figures in parentheses are % of total number in sample
Table 2.

**IFA result of the total samples tested**

*(Sera + Dried blood spots)*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>IFA test (sera + dried blood spots)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>strong</td>
<td>moderate</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td><em>B. higemina</em></td>
<td>145(47.9)</td>
<td>55(18.2)</td>
<td>103(33.9)</td>
</tr>
<tr>
<td><em>B. horis</em></td>
<td>40(13.2)</td>
<td>63(20.8)</td>
<td>200(66.0)</td>
</tr>
<tr>
<td><em>T. annulata</em></td>
<td>60(19.8)</td>
<td>45(14.9)</td>
<td>103(33.3)</td>
</tr>
<tr>
<td><em>I. tubulosa</em></td>
<td>243(80.2)</td>
<td>23(7.6)</td>
<td>037(12.2)</td>
</tr>
</tbody>
</table>

Total number in sample 303

Figure in parentheses are % of total number in sample.
and were identified at the laboratory. For transmission experiments and laboratory ticks colonise many ticks from all species prevalent at Sagoi were collected alive. The total number of preserved male and female ticks was 2294.

The highest number of the *Boophilus decoloratus* tick collected from a single animal was 16 female and this was recorded on two animals only.

6. Discussion

A preliminary quantitative survey was conducted at Sagoi area 13° 30' N 21° 6'E, and the survey included the clinical examination, post-mortem findings, microscopic examination of peripheral blood, serological studies, and the prevalent ticks. Only 5 cases (0.62%) out of 798 head of cattle were found to have babesiosis with clinical manifestations. A total of 35 cases (7.13%) were found to harbour *B. bigemina* either
Table 3.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>No. collected (male and females)</th>
<th>Average of infected on each animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyomma lepidum</td>
<td>764 (33.3)</td>
<td>4.76</td>
</tr>
<tr>
<td>Argasidium avertsi</td>
<td>460 (20.1)</td>
<td>2.66</td>
</tr>
<tr>
<td>Argasidium argineus group</td>
<td>413 (18.0)</td>
<td>2.53</td>
</tr>
<tr>
<td>Argasidium simus</td>
<td>028 (1.2)</td>
<td>0.18</td>
</tr>
<tr>
<td>Argasidium camoasi</td>
<td>068 (3.5)</td>
<td>0.55</td>
</tr>
<tr>
<td>Boophilus decoloratus</td>
<td>252 (11.0)</td>
<td>1.58</td>
</tr>
<tr>
<td>Boophilus annulatus</td>
<td>050 (2.2)</td>
<td>0.31</td>
</tr>
<tr>
<td>Hyalomma a. graticulum</td>
<td>033 (1.5)</td>
<td>0.21</td>
</tr>
<tr>
<td>Hyalomma dromedarii</td>
<td>069 (2.8)</td>
<td>0.41</td>
</tr>
<tr>
<td>Hyalomma truncatum</td>
<td>003 (0.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hyalomma rufipes</td>
<td>139 (6.0)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

a. Total number in sample 2244 ticks
b. Total number of animals 266
c. Figure in parentheses are % of total number in sample
d. average of infected ticks on each animal.
pure infection or mixed with Anaplasma spp. or Theileria spp., so 48 head of cattle had subclinical babesiosis (50.5%) of the positive 93 cases). Joyner and Donnelly 1979 stated that infection with babesial parasites is not always accompanied by readily observed symptoms, this agrees with results obtained by IFA tests which gave 66.1% positive sera for B. bigemina despite the microscopical examination revealed 7.15% and symptomatic babesiosis observed in 0.68% during the survey period.

About 1.59 Boophilus decoloratus (male and female) and 0.31 Boophilus annulatus tick were the average of Boophilus spp., collected from one animal during the survey, but FAO terminal report of the Tick and Tick-borne Diseases Control Project in Sudan 1983 reported that during the outbreak of Babesiosis in 1979, on Sagad area at the end the rainy season up to 20 Boophilus spp., ticks were found on many animals. This indicated significant variations in vector tick numbers over seasons or
years at Sagadi. When such a situation is experienced in an area according to Gunnow (1971) and Nastic (1981), a number of animals escaped exposure to babesiosis and the area is a typical marginal zone for the survival of Babesia sp. Jeynes and Donnelly (1979) reported that the incidence of the tick vector and the conditions that influence its behaviour and activity significantly affect the incidence of disease. All these assumptions can explain the reported outbreak of babesiosis 1979 at Sagadi and the possibly outbreaks may occur at Sagadi area and in other areas with the same peculiarities. The situation at Sagadi and similar areas may be further complicated by cattle arriving from the south (4° 30' to 10° 0') during rainy season (600 mm to more than 1500 mm annually) when these areas become suitable for Babesia sp. as reported by Hoogstraal (1986).
CHAPTER IV

EXPERIMENTAL TRANSMISSION OF BABESIA HINUMA

1. Introduction

Sculsby (1968) stated that the erythrocytic forms of Babesia spp. are readily transmissible by mechanical means to another animal, and these then initiate a further cycle of asexual reproduction. Callow and Hoyte (1961), Bishop et al. (1973) used the quick serial passage of infected blood with Babesia and several different organisms, as a method to separate Babesia from contaminating organisms in spleen cellular cultures. The method of separation performed by Callow and Hoyte (1961) and Bishop et al. (1973) was based on that used by Sergeant et al. (1927).

Transmission of Babesia has been reviewed or studied by, amongst others, Hoogstraal (1956),
Heitz (1956), Morraria et al. (1977) in Kenya, Pethigiser (1977) in South Africa. Pick (1964, 1968) described {species} as parasites of ticks; however, cattle reacting to tick-transmitted infections were more infective to the ticks than those infected by blood inoculation.

In an attempt to add to our knowledge of the transmission and course of infection of B. bigemina in northern Sudan, a series of laboratory investigations have been undertaken. As starting point, it was necessary to have the Sagadi field strain of B. bigemina free from other contamination organisms. Babesia bigemina carriers at Sagadi area were almost invariably infected with at least one other haemoparasite viz. A. hovis Theileria annulata, Theileria mutans and Anaplasma spp. (see chapter III).

The laboratory investigations describe how B. bigemina Sagadi strain was obtained relatively free of contaminants by blood inoculations subinoculations in clean splenectomized calves. Trials to test the
Infectivity of *Boophilus decoloratus* collected from Sagadi area were carried out. Further more, experimental pick-up and transmission attempts have been done with *Boophilus decoloratus*, *Boophilus annulatus* and *Hyalomma s. anatolicum* ticks in splenectomized calves.

2. Experimental subinoculation of infected blood onto clean splenectomized calves

Two blood stabilates were prepared at the laboratory, stabilates no 51 and 52. They were originally obtained from naturally infected cattle at Sagadi.

Stabilate 51 failed to infect a splenectomized clean calf (AS4). So, stabilate 52 was used as an original Sagadi strain and successful passages were done.

On post-mortem examination the carcass was yellowish in colour with multiple focal haemorrhages. The trachea was fleshy and so were the lungs. There were petechial haemorrhages on the tongue and intestine, ulcers on the abomasum and
The Leria spp.

A: Preparation of STA. 53

B: Treatment with ACAPRIN 5%
extra markings in the rectum. The liver was enlarged with white infarcted areas. The heart was flabby with many infarcts. Kidneys were inflamed and urinary bladder congested.

2.2. Experiment No. 2

Inoculation of stabilates 53 into calf A59 (day 0)

This was the second passage of stabilates 52. As stabilates 53 was prepared on day 4 post-inoculation from A37 which was originally inoculated with stabilates 52. Stabilates 53 was prepared by addition of 10% glycerol, 3% B. digesiana parasites were calculated in each 1000 (rbc/a) of the blood stabilates. A59 was a splenectomized clean calf inoculated subcutaneous (s/c) on the neck side with 3 ml stabilates 53 on day 0.

B. digesiana was detected on day 5 post-inoculation. Blood stabilates no. 63 and 64 were made on days 5 and 6 post-inoculation respectively.
The blood parasitaemia was about 0.6%, 1.0% *E. bigemina* on days 5 and 6 respectively. The calf unexpectedly died on day 15 post-inoculation from acute typhus, and up to that day it was positive for *E. bigemina*.

2.3. Experiment No. 2

Inoculation of stabilate 63 into
calf A55 (day 0)

A splenectomized clean calf A55 was inoculated s/c with 8 ml of stabilate 63 previously prepared from calf no. A53.

*E. bigemina* was detected on the peripheral blood for the first time on day 4 post-inoculation and stabilate 65 was prepared. 10%glycerol blood stabilate was made, the calf parasitaemia on day 4 post-inoculation was about 0.7%. The calf was treated with aspirin 5% at dosage of 0.720 ml/kg body weight in the same folds on day 16. Oxytetracycline was also injected intra-muscularly.
at dosage of 10 mg/kg body weight to prevent the possible appearance of Theileria spp. and/or Anaplasma spp. No. parasite was seen in the peripheral blood for 15 days after this treatment.

2.4. Experiment 4

Inoculation of stabilate 65 into calf 457 (day 0)

457 was a clean opharmacized calf, on day 0 it was inoculated subcutaneously with 8 ml stabilate 65. B. Diesima was detected for the first time on day 5. Stabilate 65 was made on that day, and the calf was treated in the same manner as calf 456 on day 15 post-inoculation.
3. Infectivity tests of field (Sagadi) collected Boophilus decoloratus

3.1. Infectivity test of ticks obtained from positive females

3.1.1. Vector

Larvae emerging from eggs of five B. decoloratus semi-engorged females were used. Four females were collected from two microscopically negative cattle and the fifth from microscopically B. bigemina positive cow at Sagadi. The five female ticks were positive for B. bigemina vermicules by monoclymph examination.

3.1.2. The test

The larvae of the each of the 5 mentioned B. decoloratus females were put in separate tick tubes. It is known that Boophilus spp. ticks are
one host ticks, so the larvae were fixed to the infestation belt which was tied around calf A30 (clean and splenectomized) chest and back on day 0. Whole body feeding of *Boophilus* spp. was practiced in this study.

3.1.3. Results

No *B. bifemia* parasite or any other tick-borne parasite were seen in the peripheral blood from day 0 up to animal's accidental death on day 80 post-infestation, and also no febrile response was detected.

Five sera samples were collected from the calf and tested by the IFAT technique. All sera had no antibodies against *B. bifemia*, *B. bovis*, *T. mutans* or *T. annulata*.

The engorged female adults started dropping on the pen floor on day 23 and
continued to drop up to day 39 post-infestation (see Fig. 2). A total of 
(456) females were collected (photograph 3).

3.2. Infectivity test of ticks obtained 
from B. decoloratus females not treated 
for vermigilae.

3.2.1. Vector

Nine tubes of larvae emerging from 
eggs oviposited by 9 semi-engorged 
B. decoloratus females Sagadi strain 
were prepared. Haemolymph of all ticks 
was not examined for vermigilae of 
B. bigemina. Each tube contained 
about 800 larvae.

3.2.2. The test

A49 was a clean intact calf with no 
antibodies against B. bigemina, B. bovis, 
Theileria mutans or T. annulata.
FULLY ENGORGED AND DETACHED FEMALE BOOPHILUS DECOLORATUS TICKS FROM CALVES (A 49) AND (A 50)

--- DETACHMENT FROM CALF (A 49)
--- DETACHMENT FROM CALF (A 50)

FIG. NO. 2
The 9 tubes of larvae were applied on the animal by the infestation belt. The application was done as follows, on day 0 three tubes, and 2 tubes on day 1, 2 and 3 respectively were applied.

3.2.3. Koculie

The calf was kept under close observation from day 0 up to day 58 post-infestation, during this period no *B. bigemina* or any other blood parasite was detected microscopically. No febrile response was recorded and no antibodies against *B. bigemina*, *B. bovis*, *T. annulata* or *T. mutans* was detected on IFA test of the 9 collected sera.

A total of 369 engorged females were harvested during the period from
day 24 up to day 42 (see Fig 2), although 4 applications were made on 4 consecutive days starting on day 0.

4. Laboratory transovarian transmission of B. bigemina by Boophilus decoloratus

4.1. First transmission

4.1.1. Pick-up by adults

4.1.1.1. Vector

B. decoloratus free from B. bigemina was used. Ticks were originally collected from Sagadi and fed for one generation on a clean susceptible calf L43 (see 3.2.) and also the females were negative for B. bigemina venereal; so these were considered clean.
PICK-UP OF B. BICEMINA BY BOOPHILUS DECOLORATUS ADULTS
USING CALF A56 (Splenectomized)

A inoculation of Stabilate No. 65.
B calf sprayed with Vapona.

PARASITAEMIA / 1000 RBCs
--- no. of fully engorged and detached B. d.

DAYS

FIG. NO. 3
in its peripheral blood till day 106 post-inoculation i.e. day 131 post-first-larval-application. No other blood parasite was detected on the peripheral blood up to day 131 post-inoculation on which the calf leg was accidently body broken and died.

4.1.1.3. Tick recovery and examination of haemolymph

Engorged females of *B. decoloratus* started dropping on day 25 post-first-application and continued up to day 53 and the calf was sprayed on day 54,55 102 engorged females were collected and labelled starting from 1. 38 engorged females dropped during days 25 to 29 post-application.

34 of the engorged females completed the oviposition, the rest either died before or during the oviposition. Only
21 of the successfully completed oviposition were found positive for *E. biscanica* vermicales after hemolymph examination (see photomicrograph 4).

4.1.1.4.  
**IHA test result**

Antibodies against *E. biscanica* only were detected on day 180 post-inoculation, the titre continued increasing up to \( 1 \times 10^3 \) on day 30 post-inoculation antibodies other than that detected for *E. biscanica* were not read till day 106 post-inoculation of stabilates.

4.1.2.  
**The transmission**

4.1.2.1.  
**Vector and host**

Larvae obtained from females that were positive for vermicales, were used. A spleenectomized olenx and susceptible
Engorged *Borrelia decolorans* (male and female, copulating) infesting calf A50 few days before detachment.

**Photomicrograph A.**

Giemsa-stained hemacytaph smear of *Borrelia decolorans* female, (10 days after detachment from calf A50). Magnification: 3600.

A. Developmental stages of *B. burgdorferi* verniculosa.

B. Mature verniculae, showing red-staining cap.
4 were applied on day 1. Each tube contained about 1000 larvae.

4.1.2. Results

A. biceps was detected microscopically on day 23 post-first-application of *Boophilus decoloratus* larvae, and continued up to day 50 on that day the calf was treated with Acephen 5% (0.720 mg/kg body weight) injected in the tail fold, (see photomicrographs 5 and 6). Temperature rose up to 41.4 °C on day 26 post-first-application and bloody urine was observed on day 30, (see Fig. 4).

4.1.2.3. Tick collection and rearing

*Boophilus decoloratus* emerged females started to leave the calf on day 22 post-
calf no. 161 was used. 4 tubes of larvae were applied on day 0 and other 4 were applied on day 1. Each tube contained about 1000 larvae.

4.1.2.2. Results

*B. higasii* was detected microscopically on day 23 post-first-application of *Boophilus decoloratus* larvae, and continued up to day 50 on that day the calf was treated with Aceprin 5% (0.720 mg/kg. body weight) injected in the tail fold, (see photomicrographs 5 and 6). Temperature rose up to 41.4 °C on day 26 post-first-application and bloody urina was observed on day 30, (see Fig. 4).

4.1.2.3. Tick collection and examination

*Boophilus decoloratus* engorged females started to leave the calf on day 22 post-
TRANSMISSION OF *Babesia bigemina* BY THE TICK *Boophilus decoloratus* USING CALF NO. A 61 (Splenectomized)

--- FULLY ENG. AND DETACHED *Boophilus decoloratus*

PARASITAEMIA

A : COLOURED URINE

FIG. NO. 4
Photomicrograph 2

Giemsa - stained blood film from calf A61, showing various forms of Babesia bigemina in the calf erythrocytes - Magnification: X 3600

Photomicrograph 6

Giemsa - stained blood film from calf A61, showing Babesia bigemina in the calf erythrocytes - Magnification: X 3600.

A. Two paired pyriform parasites in one erythrocyte.

B. One paired pyriform parasite in erythrocyte, normo.
application of larvae and continued up 18 May 51. 129 S. m. m. were harvested.

89 females were found dead either before or during the completion of oviposition and of the rest 51 were positive for B. hislopina vermiculus (see photomicrographs 7 and 8).

- It is worth reporting that all engorged females died before or during oviposition in all transmission records here, their haemolymph was red in colour.

4.1.2.4. (EPA) test positive

When the calf was microscopically positive, sera were taken daily for the EPA test. Antibodies against B. hislopina were detected for the first time on day 4 post parasite appearance in peripheral blood and a titre of 160.
A. Incubate vermicules (red-capped vermicules)

B. Haemolymph cell.

Photomicrograph 2

Giemsa-stained haemolymph smear of *Boophilus decoloratus* female, (10 days after detachment from adult), Magnification x 3600.

A. Spherical body ready to elongate and become a vermicule

B. Mature vermicule

C. Haemolymph cells.
was reported. One week later a titre of 1:30 was recorded. No antibodies other than that for B. bipalum were detected.

4.2. Second transmission

Another attempt to transmit B. bipalum by Boophilus decoloratus ticks transovarially was done.

4.2.1. Yeast

10 plastic tick tubes containing Boophilus decoloratus larvae obtained from females with vermiform in the haemolymph were used. These females dropped from calf A61, (see 4.1.2.3.). One day feeding was performed this time and 16 tubes of larvae (each contained about 800) were fixed on two infestation belt and tied around the calf chest on
day 0.

4.2.2. The transmission

A splenectomized clean susceptible calf no. AT3 was used. The parasite E. bigemina was detected for the first time on day 28 post-application by microscopic examination. The parasites continued to be detected microscopically up to day 62, and on that day the calf was found dead possibly due to enzootic bite.

The first Haemaphysalis decolorata engorged female was collected on day 28, and continued to drop up to day 35 post-application. Only 34 females were collected from the floor this time.

On examination of the haemolymph only 10 were found positive for cercariae, and none of them died. Calf AT3 did
not show any clinical symptoms despite being splenectomized. None of the three calves A56, A61 and A73 died due to babesiosis.

5. Laboratory attempts to transmit *B. bigemina*
   by *Hyalomma prostratum* (H. a. a.)

5.1. First trial
5.1.1. Flux-up

*Hyalomma a. australicum* s.l. batch, Sheshat strain (Khartoum Province) were used. All instars larva, nymph and adult were applied for pick-up. About 1000 larvae, 500 nymphs and 20 females and 20 males were applied on each calf. Two calves positive for *B. bigemina* due to blood inoculation, calf A59, 459 (see 2.1. and 2.3.) were used.

The engorged ticks were then collected by shaking the ear-lung and
each instar was placed separately in tubes. The engorged adults females were separated singly each in a tube. All instars incubated at 28 °C.

3.1.2. Transmission attempts

For each emerged instar separate clean and splintereized calf was used.

Larvae emerging from H. E. E. adults that fed on calves A55 and A59 were applied to calf A74 on day 0. No febrile response or B. bigemina parasite in blood were detected up to day 86 post-application.

Emerging adults were fed on calf A63 and observed as in the case of calf A74 but the same result was obtained up to day 30 post-application. No antibodies were detected against B. bigemina, T. mutana, or.
in both calves 674, 653 were
had been tested.

Trypsin resulting from larvae fed
on calves 455 and 459 (T. bicinamia
positive calves), were applied on
calf 463 on day 0. T. bicinamia was
detected macroscopically on day 26
post- application and temperature
reached 40.2 °C on day 30. After day
35 the animal started to recover and
showed irregular presence of T. bicinamia
in its peripheral blood. It was
treated with Aspirin 5% on day 40
post- application.

5.2. Second trial

5.2.1. Pick-up

This time, calf 461 which was a
Tick- transmitted T. bicinamia-
parasitic mic- animal was used for pick-
(see 4.1.2). About 1000 larvae, 100 nymphs and 20 males and 20 female adults were applied to the ears of calf A61 during the parasitoxic phase.

5.2.2. The transmission attempt

Clean splenectomized calves were used for each instar resulting from the pick-up trial.

For resulting larvae calf A75 was used, but no transmission was achieved; the resulting nymphs of A.A.G. from the calf A75 were also applied on calf A76 but also no transmission was achieved.

Resulting nymphs and adults obtained from the larvae and nymphs that were applied for pick-up on A61 were applied on calves no A77 and A78 respectively. No transmission was achieved in any calf.
and no antibodies were detected in the
two calves for 50 days post-application.

5. Laboratory trials to transmit B. bigemina
by Boophilus insignitus.

Two species of Boophilus insignitus obtained
from Sagadi and Kadogo (11° 30’N 29° 35’E) were
collected as semi-engorged females and reared at
the laboratory at Sola, Arusha Province. The
larvae of each strain were applied on separate clean
splenectomized calf, during summer. None of the
larvae of both strains was attached to the animal
skin or dropped up to day 50 post-application.

7. Discussion

Four passages of mixed infected blood were
made in about two months in an attempt to have
a relatively pure infection of B. bigemina. Gallot
and Hoyte (1961) did five passages with fresh
infected blood in eight days. The four passages
are same results observed by Callow and Hoyt (1961), because on the first day that \( B. \) bigemina was detected microscopically, a 1:6 glycerol infected blood stabillate was made and whenever another calf was ready, the prepared stabillate was inoculated. It was found that \( B. \) bigemina needed 4-5 days to be detected microscopically in blood smear and by the fourth passage a relatively clean stabillate no. 65 was obtained. Either the Thelidopa spp. and Anaplasm spp. do not multiply as rapidly, or their predilection site is the visceral circulation, these were the still valid explanations for the early detection of \( B. \) bigemina, as was suggested by Callow and Hoyt (1961). Sergeant, et al. (1927) observed an increase in \( B. \) bigemina virulence after serial rapid passages, but neither Callow (1961) nor this study was able to notice any increase in virulence, possibly because of the techniques and small number of calves used.
Boophilus decoloratus females collected from Segadi and positive for venous blood of E. bigemina failed to infect the splenectomised calf; possibly the infection inocula of the second generation was very low to initiate detectable parasitaemia or antibodies on the IFI test.

Two successful E. bigemina transmissions with Boophilus decoloratus nymphs were performed. It is possibly the natural vector of this parasite in northern Sudan. The same conclusion was suggested by Morgaria (1977) in Kenya.

Unsuccessful attempts were conducted on the progeny of two batches of Boophilus annulatus to test their infectivity, but no attachments were detected. The failure can be attributed to the fact stated by Morgaria (1956) that this tick survived in an annual rain fall of 600 - 1500 mm and high relative humidity, or might be due to the techniques used. Need for suitable techniques should be kept
Transmission with H. pulchra tick resulted in a positive doubtful transmission by the nymph in one attempt and failed in the second attempt, the larvae and adults failed to transmit the infection twice. H. pulchra was incriminated in H. longicornis transmission according to Indian workers, Chaudhri et al., (1975) and unidentified Babesia spp. was also transmitted by Hyalecta mesostoma pulchra (Thomson and Mason, 1931). Evidence indicated the possibility that the Hyalecta spp. may transmit Babesia spp., as the role of Hyalecta spp. and possibly Hyalomma spp. should be further investigated and with better techniques to explore their possible role in the epizootiology of bovine babesiosis in Sudan.
CHAPTER V

CONCLUSIONS

The first recognition of babesiosis in cattle in Sudan was reported by Balfour (1905) and the identification of the species responsible was later done by many veterinarians in many parts of the Sudan (Animal Wealth Administration Reports, 1960-1981) and Prof. Karib Personal Communications, 1983). The one species reported before (1979) was *B. divergens* but lately the FAO-Tick and Tick-borne Diseases Control Project, Sudan identified a second species *B. bovis* (Project Terminal Report, 1983). In an attempt to gain more knowledge of *B. divergens*, the commoner species in Sudan, and to some extent *B. bovis* the second species believed to cause babesiosis in Sudan a preliminary survey was conducted at Sagad area where the FAO-Tick and Tick-borne Diseases Control Project reported the presence of both
disease picture, but still it was a landmark indicating the need for long-term epidemiological studies in other parts of Sudan.

Babesiosis in Sagadi during the survey period was not a problem, but still the possibility of a second outbreak as that reported in 1979 must be considered. Although blood smears examination gave positive results of not more than 5%, the IFA test positive cattle were 66.1% and the *Babesia sp.* infection averaged about 2 ticks per head. These results are not in agreement with the conclusions expressed by Mahoney and Ross (1973) who stated that in places where ticks are numerous the epidemiology of babesiosis is characterized by stability implying a high incidence of organisms in cattle but rarely the occurrence of clinical disease; while investigation survey at Sagadi indicated instability of babesiosis so that seasonal outbreaks of the disease could
occur as a result of increased number of tick(s).

A new record of *T. bispinosa* was identified and confirmed by Clifford and Wessell working with Hoogstraal at Naval Medical Research Unit (NMURU) No. 3 Cairo on 30 Sept. 1962, the species concerned was *T. bispinosa canicola*. This is the first recognition of the presence of this species in the Sudan.

The method of detection of antibodies to *B. microti* in dried blood samples using the IFA test, adopted by Burridge et al. (1973), was used successfully not only for *B. microti* but for *B. bovis*, *Theileria mutans* and *B. anaplasma* in Sudan. Todorovic and Garcia (1973) made a comparison of the dried blood on filter paper and serum techniques for diagnosis of babesiosis experimentally and under field conditions utilizing the (IFA) test, they found no significant differences in the sensitivity and specificity of both techniques. Their conclusion was that the use of dried blood may be a valuable aid for the epidemiologic studies of *Babesia spp.*
clear B. bigemina after which the calves used were treated.

One experimental pick-up of B. bigemina was
by Boophilus decoloratus and subsequently two
transovarian transmissions were achieved. It is of
interest to note that the prepatent period of B.
bigemina on the first transmission was 23 days and
the detachment of females began on day 22 post-
first-larval-application, while on the second
experiment the prepatent period was 21 days post-
application of larvae and females drop began on
day 28 also. Callow and Hoyte (1961) recorded
that one of their calves showed a prepatent period
of B. bigemina transmitted by Boophilus micropilus
nymphs of 28 days, but they added that the prepatent
period of this organism when transmitted by ticks,
parasitic life cycle on different seasons of any tick is fundamental for accurate studies of diseases transmitted by them. The transmission of *B. bimini*, via *Boophilus decoloratus* nymphs is tabulated on table A.

Infection rate in the repleted females of *Boophilus decoloratus* was done by hemolymph examination of all such ticks from calves 456, 461, and 471. Risk, (1968) reported that the density of parasites in the blood of the bovine host following tick transmitted infection has an important bearing on the infection rate of replete females. About 30% of female ticks detached from animals with parasitemia (about 20% of these), died within 7 days and their hemolymph became red. The surviving laid few eggs. This is in contrast within the case of replete ticks dropping from calves infected with direct blood inoculation. Results obtained in this study agreed with the idea of Risk (1968), but despite
Table 2

The transmission of E. bieneusi by Rhipidomus delatensis ticks using clean splenectomised calves

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Approx. number of applied larvae</th>
<th>Prepatent period in days</th>
<th>Max. Temp. reached</th>
<th>Max. Proliferation observed</th>
<th>Other symptoms</th>
<th>Experimental calf fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A61 1st trans</td>
<td>8000</td>
<td>23</td>
<td>41.4°C (day 26)</td>
<td>40/1000</td>
<td>Bloody diarrhea (day 30)</td>
<td>treated with Acetaminophen (day 30)</td>
</tr>
<tr>
<td>A73 2nd trans</td>
<td>1000</td>
<td>28</td>
<td>normal below 39.6°C (day 30)</td>
<td>5/1000</td>
<td>none</td>
<td>Artificial death (day 32)</td>
</tr>
</tbody>
</table>

Days were post-first-larval application.
Table 2

Death and Infection Rate in R Hodgkiss Female Mice

<table>
<thead>
<tr>
<th>Calf</th>
<th>Method of transmission</th>
<th>Blood or</th>
<th>blood</th>
<th>number of</th>
<th>number of</th>
<th>number of</th>
<th>number of</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>Blood</td>
<td>8 ml.</td>
<td>91.9</td>
<td>52</td>
<td>102</td>
<td>91 (94.3)</td>
<td>84 (92.3)</td>
</tr>
<tr>
<td>161</td>
<td>ticks</td>
<td>6000 LL</td>
<td>123</td>
<td>62 (52.0)</td>
<td>51 (73.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>473</td>
<td>ticks</td>
<td>8000 LL</td>
<td>34</td>
<td>34 (100)</td>
<td>10 (29.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the very low parasitaemia produced, some ticks were found dead usually within 7 days after detachment.

Transmission of *B. bigemina* by *Boophilus annulatus* failed in two attempts, but further trials should be conducted specially in areas with high incidence of this species of ticks.

The successful transmission of *B. bigemina* by *Rhipicephalus s. appendiculatus* nymphs in one experiment and the failure in another may indicate the possibility of other vectors of *Babesia* in Sudan, especially in northern areas with incidence of *Babesia* in cattle (Animal Health Annual Reports 1960-1971) and very low or no presence of *Boophilus* spp., according to Hoogstraal (1956). The role of *Boophilus* spp. as a natural vector of *B. bovis* and *B. bigemina* and possibly other bovine Babesias have been determined after intensive experimental studies done by a number of workers notably Mahoney (1977), Kick (1964, 1968), Heitz (1956). Very intensive experimental and natural studies should be
conducted to determine the role of any of other species of ticks in transmitting Schagid spp. to cattle especially *Hyalomma anatolicum anatolicum*, *Rhipicephalus evertsi evertsi* and *Haemaphysalis pulicaria*. 
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