

# Research Note on Ticks and Tick-Borne Diseases of South Eastern Tanzania

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## Abstract

Tick and blood surveys were carried out in the Lower Kihansi area in Iringa and Morogoro Regions of South-eastern Tanzania. The objective was to identify the tick species present in the area and isolate the tick-borne pathogens transmissible to man and animals. This was in response to complaints from non-indigenous people working in the area who got bitten by ticks and suffered serious reactions. The tick species identified from the area were *Amblyomma variegatum*, *Rhipicephalus appendiculatus*, *R. evertsi*, *R. kochi*, *R. pravus*, *R. sanguineus* and *Haemaphysalis leachii*. Efforts to isolate the pathogens from ticks failed but serological tests by use of Enzyme Linked Immunosorbent Assay (ELISA), Immunofluorescent Antibody Test (IFAT) and Hemagglutination Inhibition (HAI) techniques on sera collected from humans, baboons, gazelle, cattle, sheep, goats, pigs, chicken and rodents, revealed the following pathogens to be circulating within the human and animal populations: protozoans: *Trypanosoma*; rickettsia: *Rickettsia conorii*, *Anaplasma* and *Cowdria*; viruses: *Wesselsbron*, *Chikungunya*, *Sindbis*, *Yellow fever* and *West Nile*. This was a preliminary survey to provide base-line information. There is need of carrying out a more extensive survey in order to establish the extent of the problem, and also to associate the different pathogens to specific tick species so as to be able to design appropriate control measures.

**Key words:** Ticks, tick-borne diseases, pathogens, south-eastern Tanzania

## Introduction

Ticks surpass all other arthropods in number and variety of diseases, which they transmit to domestic animals, and rank second to mosquitoes as vectors of human diseases.

There are eight tick genera with 60 identified species and two unnamed species in Tanzania (Yeoman and Walker, 1967). However, only three genera with nine species/varieties are known to be of economic importance to cattle. Ticks and tick-borne diseases (TBD) are responsible for causing over 65% of the total annual cattle mortality in Tanzania, with annual losses estimated to be US \$ 5.1m (Kagaruki, 1997). There is no published information regarding ticks and TBD of hu-

mans in Tanzania. This is probably because, the majority of TBD of humans are non-specific in their initial clinical and laboratory presentation, and, may be confused with a variety of more common illnesses. Most of these diseases also do respond to the common antibiotics, consequently, they are normally treated without knowledge. However, late or lack of treatment of some of the TBD of humans, can result in serious consequences and sometimes death (Doan-Wiggins, 1991).

Clinical features of the human tick-transmitted pathogens range from mild fevers, headache, myalgia and gastrointestinal involvement, to fatal haemorrhagic fevers (Ansari and Shope, 1994; Gear *et al.*, 1990; Yagupsky

and Wolach, 1993). Cutaneous B cell lymphomas have also been associated with tick-transmitted pathogens, with skin tumours persisting for up to 7 to 15 years (Garbe *et al.*, 1991).

Recently, cases were reported by non-indigenous workers in the Lower Kihansi Hydroelectric Power Project, in Morogoro and Iringa Regions, who got beaten by ticks and suffered serious reactions. These reactions ranged from lymphadenopathy, fevers, headaches and myalgia, some of which persisted for several months (Kim Howell, personal communication).

On realising the importance of TBD, and the lack of necessary information concerning both these diseases and their vectors in the Lower Kihansi area, a study was designed to identify the tick species within the project area; to find out whether those ticks carry any pathogens dangerous to man and animals and whether those pathogens are circulating within the human and animal populations.

## Materials and Methods

### Study area

The Lower Kihansi Hydroelectric Power Project is located in south-eastern Tanzania, approximately 85-km south-east of Iringa Region, at 35°52' E and 8°14' S across the boundary of Morogoro Region; to the Udzungwa plateau in Mufindi District, Iringa Region. It lies on the Kihansi River, a tributary of Kilombero and Rufiji Rivers. The area is also surrounded by forest reserves namely, Udzungwa, Njerera, Idewa and Ihagana, which form an important part of the Kihansi river catchment. The area was divided into three main parts, namely, Mlimba and Uhafiwa wards, and the forest area. Each part had four transects and each transect had eight sampling points, all randomly selected.

### tick Collection

Ticks were collected from 31 cattle, 126 goats, 20 sheep and 2 rodents only, as the other animals (gazelles, a baboon, pigs, chicken, duck, pigeons and rodents) did not have any ticks on their bodies. Due to the small number of livestock in the area, all the animals at each point were sampled.

Cattle were thrown down mostly by snaring the hind legs and then restraining them. The other

livestock were either grabbed and sampled while standing, or restrained to the ground by using bare hands. The gazelles and baboon were shot to the ground, while the rodents were trapped using the conventional Sereñgeti trap (Senzota, 1987).

Ticks were collected from the animals using the method described by Yeoman and Walker (1967). After collection, the ticks were stored in special tick containers with moistened cotton wool to prevent desiccation. Ticks were also collected from the pasture by use of the drag method described by Sutherst *et al.*, (1978); Norval *et al.*, (1987) and Georgi, (1974). The ticks were later removed from the drag and stored in tick containers. The drag method could not be applied in the forest area due to the nature of the terrain and vegetation found there.

After collection, the ticks were identified by using a stereo microscope and an identification key. After identification, the ticks were placed in properly labelled cryopreservation tubes and stored in a 5-litre liquid nitrogen tank, which was refilled weekly. All the ticks belonging to similar species and from the same area were pooled together in identifiable canisters.

### Blood collection

Blood was collected from livestock namely cattle (30), sheep (35), goats (70), pigs (25), chicken (69), duck (1) and pigeons (2) and also from wildlife namely rodents (20), gazelles (2) and a baboon (1). Blood was drawn from the jugular vein in the case of cattle, sheep and goats, using sterile needles and vacutainer tubes. In the case of gazelles and baboon, blood was drawn from the wound immediately after shooting, using a 5ml syringe and later pushed into the vacutainers. A 5ml syringe was also used to draw blood from pigs (from the tail or abdominal vein), chicken, ducks and pigeons (from the wing vein). In rodents, blood was drawn from the heart, using a 5ml syringe. Blood was also collected from 10 people from the radial vein using sterile needles and vacutainer tubes.

The vacutainer tubes containing blood were stored in a dry place away from direct sunlight, until the next morning. Early in the morning, sera which had by then separated was decanted from the vacutainers into labelled bijou bottles and stored at -20°C. Both the ticks and blood

were finally transported respectively in liquid nitrogen container and in ice - cool box to the Animal Diseases Research Institute (ADRI) laboratory in Dar es Salaam.

### Pathogen identification from sera

A total of 265 sera collected from the study area was sent to the Onderstepoort Veterinary Institute and the National Institute for Virology in South Africa for pathogen identification.

At Onderstepoort, the animal sera was tested by use of the enzyme linked immunosorbent assay (ELISA) and immunofluorescent antibody test (IFAT), against viruses: (*Borrelia burgdoferi*, Rift Valley and Wesselsbron); rickettsia: (*Cowdria* and *Anaplasma*); and protozoa: (*Trypanosoma*).

At the National Institute for Virology, ELISA, haemagglutination-inhibition (HAI) and immunofluorescence (IF) techniques were used to screen human and baboon sera against virus antibodies to Crimean Congo Haemorrhagic Fever (CHF), Bunyamwera, Dugbe, Nairobi Sheep Diseases (NSD), Sindbis, Chikungunya, Wesselsbron, West Nile, Rift Valley and Yellow Fever, as well as against rickettsia (*Rickettsia conorii*).

### Virus and rickettsia isolation from ticks

At the ADRI laboratory, the ticks were removed from the liquid nitrogen container, pooled together according to tick species from the same or close geographical area. All the tick pools were washed immediately, three times with sucrose phosphate glutamine buffer and then ground in a sterile mortar with sterile sand and centrifuged at 3000 rpm for ten minutes. The clear supernatant fluid was divided into two aliquots; one for virus and the other for rickettsia isolation.

### Virus Isolation Using Mice

Virus isolation was made using two to three days old suckling white Swiss mice obtained from the Rodent Research Project at the Sokoine University of Agriculture, Faculty of Veterinary Medicine, Morogoro, Tanzania. Prior to inoculation, the tick extract samples were diluted with 50% v/v Borate Albumin Buffered Saline pH 7.0, containing antibiotics (penicillin 1000 i/u per ml and streptomycin 2.0 mg/ml).

The suckling mice were each inoculated with 0.03 ml of the diluted supernatant intracerebrally. The mice were closely observed for a minimum of 14 days for signs of illness. Any mice which showed signs of illness had its brain harvested and passaged into fresh litter according to the technique by Hsiung (1973).

### Rickettsia isolation using eggs

The tick extract aliquots intended for rickettsia isolation was inoculated into six days old embryonated eggs through the egg yolk route. Each egg was inoculated with 0.25 ml of the supernatant. The eggs were incubated at 34°C and candled daily to detect any deaths. Eggs dying within two days were considered non-specific (Hsiung, 1973).

## Results

### Tick identification

Table 1 shows the tick species collected from different sites within the project area and the hosts from which they were collected. Ticks collected from the different areas were as follows: Mlimba Ward: *Amblyomma variegatum*, *Boophilus microplus*, *Rhipicephalus appendiculatus*, *R. evertsi*, *R. kochi*, *R. punctatus*, *R. sanguineus* and *Haemaphysalis leachii*. Forest area: *Amblyomma* nymphs, *R. evertsi* and *R. sanguineus*. The dog ticks and also vectors of *R. conorii* i.e. *Rhipicephalus sanguineus* and *Haemaphysalis leachii* featured in all the three surveyed areas. *Amblyomma variegatum*, a suspected vector of *R. conorii*, was also found in high frequencies in two of the three surveyed areas namely Mlimba and the forest area.

### Pathogens Identification by Serology

Table 2 shows the different pathogens identified from the tested sera. These pathogens included: viruses - Wesselsbron, Sindbis, Chikungunya, West Nile, and Yellow fever; rickettsia - *Rickettsia conorii*, *Anaplasma* and *Cowdria*; and protozoa - *Trypanosoma*.

Table 1: Ticks Identified in the Lower Kihansi Hydroelectric Power Project Area

Area	Host	Tick Species
Mlimba	bovine, caprine, ovine, canine, rodent, pasture	<i>A. variegatum</i> , <i>B. microplus</i> , <i>H. leachii</i> , <i>R. appendiculatus</i> , <i>R. evertsi</i> , <i>R. kochi</i> , <i>R. pravus</i> , <i>R. sanguineus</i> <i>Rhipicephalus</i> nymphs
Uhafiwa	Canine, caprine, pasture	<i>R. sanguineus</i> , <i>Rhipicephalus</i> nymphs, <i>R. pravus</i> , <i>H. leachii</i>
Forest	canine, rodent	<i>Amblyomma</i> nymphs, <i>R. evertsi</i> , <i>R. sanguineus</i>

Table 2: Pathogens Identified in the Lower Kihansi Hydroelectric Power Project Area

Pathogen	Host	Test	% Positive	Titre levels
<i>R. conorii</i>	human	IF	38.5	1:128 - 1:2048
Wesselsbron	cattle	IFAT	63.3	1:700 - 1:18800
	goats	IFAT	60.4	1:1900 - 1:18900
	sheep	IFAT	50	1:1300 - 5200
Sindbis	human	HAI	8.0	1:20
Chikungunya	human	HAI	23.08	1:20 - 1:80
	baboon	HAI	8.0	1:20
West Nile	human	HAI	15.39	1:40
Yellow fever*	human	HAI	8.0	1:20
Anaplasmosis	cattle	C-ELISA	26.6	NG
Heartwater	cattle	Ab ELISA	40	NG
Trypanosomiasis*	cattle	Ab ELISA	36.7	NG

\* Transmitted by mosquito

\* Transmitted by tsetse

The Wesselsbron virus which was identified from bovine, caprine and ovine was the most common virus detected in livestock sera as follows: bovine - 63.3%, caprine - 60.4% and ovine - 50%. The antibody titres in the positive animals were as high as 1:18800 in bovine; 1: 15200 in caprine and 1:13700 in ovine. Anaplasmosis, heartwater and trypanosomiasis also featured strongly in the bovine, with 26.7%, 40% and 36.7% of the population affected respectively. For the human sera, *R. conorii* was also common with five out of the 13 samples testing positive. The antibody titre levels ranged between 1:128 to 1:2048. For the viruses, the antibody titre levels were however low ranging from 1:20 with the Sindbis virus, to 1:80 with the Chikungunya virus

### Virus isolation in mice

A total of seven samples were suspected of being virus positive. These were passaged in mice three times, but in the end, none proved to be unequivocally positive.

### Rickettsia isolation in eggs

Over 90% of the eggs originally inoculated with tick extract showed heavy bacterial contamination which precluded further investigation. Attempts were made to examine the bacterial contaminated eggs and those samples found to be less contaminated were passaged in fresh eggs after diluting the supernatant to 1/100. In spite of this, bacterial contamination was dominant and continued to kill the eggs within two days. Three of the samples killed the eggs after three days and did not appear to suffer from bacterial contamination. The yolk sac of these samples were harvested for further passages. However, smears made from these samples did not indicate any presence of rickettsia either. No further work was done on these samples.

### Discussion

Nine tick species were collected from the Lower Kihansi Hydroelectric Power project area namely, *A. variegatum*, *B. microplus*, *H. leachi*, *R. appendiculatus*, *R. evertsi*, *R. kochi*, *R. pravorus*, *R. punctatus* and *R. sanguineus*.

Species of the genus *Amblyomma*, *Rhipicephalus*, *Boophilus* and *Haemaphysalis*

have been associated with different tick transmitted pathogens, both to man and animals (Kelly and Mason, 1990; Kelly *et al.*, 1994; Morrill *et al.*, 1991 and Gear *et al.*, 1990).

From the serological results, it can be seen that the Wesselsbron virus which was identified from bovine, caprine and ovine sera was more common, with high antibody titres in all the three species of animals. This virus is known for causing a non-fatal, influenza-like illness in humans (R. Williams, personal communication). The virus is also known for causing epizootics in sheep, giving rise to abortions and deaths in new-born lambs. It can also cause abortion in cattle (Andrewes *et al.*, 1978). This virus was not identified in any of the human sera tested, indicating that probably the virus is not a problem in humans in Tanzania.

However, other viruses namely, Sindbis, Chikungunya, West Nile and Yellow fever were detected in human sera. It is possible that these viruses were responsible for ill-health complaints ranging from fever, joint pain, headache, myalgia and lymphadenopathy reported by individuals who were bitten by ticks in the study areas. Chikungunya virus was also identified from the baboon sera. The yellow fever sero-positive was detected in one person who had travelled abroad, indicating that the positive reaction could be caused by vaccination against the Yellow Fever virus, as is routinely required of people travelling abroad. This virus is normally transmitted by the *Aedes aegypti* mosquitoes which have been reported to be present in the study area (Lyimo *et al.*, 1995)

*R. conorii* was identified from five of the 13 human sera. This rickettsia which is a member of the spotted-fever group (SFG) rickettsias, is the causative agent of tick-bite fever in Africa and Boutonneuse fever in the Mediterranean regions (Kelly and Mason, 1990). In Zimbabwe, a pathogenic SFG rickettsia was isolated from a patient bitten by ticks who suffered from fever, headache and regional lymphadenopathy (Kelly *et al.*, 1994). These are similar symptoms that were felt by individuals who were bitten by ticks in the study area.

*Cowdria ruminantium*, a rickettsia transmitted by *Amblyomma* ticks, was detected in 12 out of 30 cattle sera that were tested. The results indicate that heartwater is a problem in the area.

*Tyrpanosoma*, which is normally transmitted by tsetse flies was detected in 36.7% of the cattle sera. Although earlier studies (Kirmse and Taylor-Lewis, 1978) indicated that trypanosomes would survive in both soft and hard ticks for varied periods, it was later concluded that ticks do not appear to play a major role in the transmission of trypanosomiasis.

There is no published information on tick-bite fever or other rickettsial and arboviral tick-borne infections in Tanzania. This report is thus the first, to show that these pathogens do exist in this country. It is unfortunate that these pathogens could not be associated with their vectors, as this association is important when designing appropriate control measures.

All the same, work done in other areas does indicate that ticks similar to those collected from the study area, are capable of transmitting pathogens to both humans and animals (Konstantinov, 1990; Kelly *et al*, 1994; Lange *et al*, 1992; Kelly and Mason, 1990; Dupont *et al.*, 1994;).

Since these pathogens cause diseases that resemble other common illnesses both in their laboratory diagnosis and clinical symptoms (Doan-Wiggins, 1991), it is important to suspect a tick-borne disease in anyone who feels sick and has a history of a tick bite. However, it should always be remembered that sometimes there have been cases of tick borne diseases in humans, without them recalling a tick bite (Yagupsky and Wolach, 1993).

## Recommendation

It would not be appropriate at this time, to spell out concrete mitigation measures against the ticks and the tick-borne pathogens within the study area, due to the limited information available. There is need of carrying out a more extensive survey, to enable realisation of the exact tick species present in the area, the pathogens they carry and the magnitude of the diseases in humans and animals caused by these pathogens.

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