Natural history of *Ornithodoros* & Vector Control of Tick-Borne Relapsing Fever in Tanzania

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BSc (Hons) MSc

Thesis submitted in accordance with the requirements of the University of Liverpool for the Degree of Doctor in Philosophy

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Dedicated to my family

&

People who are affected by the disease

"I shared experience of the tragic agony with those affected much with the disease in the region when for the first time in my life got infected with Borrelia pathogens while executing this work in the field in 2004"

DECLARATION

This work has not been submitted in substance for any other degree nor is it being currently submitted in candidature for any other degree.

Signed---------- ---------- (Candidate)

Date---------- 17th November 2006 ----------

Statement 1

This thesis is the result of my own investigations, except where otherwise stated. All other sources are acknowledged and a bibliography is appended.

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I also hereby give consent for my thesis, if accepted, to be available for photocopy and inter-library loan and for the title and abstract to be made available to outside organisations.

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ABSTRACT

Tick-Borne Relapsing Fever (TBRF), caused by *Borrelia duttonii* and transmitted by soft ticks of the *Ornithodoros moubata* complex, is a widespread endemic human disease in Tanzania. Although national statistics do not yet exist, it is clearly of major public health importance and in certain regions it is known to be an important cause of maternal and child morbidity and mortality. The disease has been neglected for decades. Notably, little research on the vectors or disease epidemiology has been carried out in recent years and much still remains unknown. Between 2002 and 2005, this study investigated aspects of the natural history and behaviour of *O. moubata s.l.* infesting households in TBRF endemic villages in Dodoma region, central Tanzania. A study was carried out to determine the knowledge, attitudes and practices of the population in relation to ticks and TBRF and the efficacy of insecticide-treated bed nets (ITNs) for vector control of TBRF was investigated in a randomised controlled trial.

To examine houses for ticks, a sampling protocol and method for distinguishing tick stages were adopted. Ticks were most abundant in floors, and tick distribution was significantly higher ($p<0.01$) in bedrooms (75.9%) than in the other parts of the house [seating areas (13.2%), kitchen (3.8%) and poultry areas (7.1%)]. Host seeking began at 21:00 hours, peaked at midnight (2400 hours) and fell gradually until 0600 hours, and was significantly higher in dry than in wet seasons ($p<0.001$). In a mark-recapture experiment, 26% of ticks released in pigpens were recaptured. Of these, 2.6% were found inside human bedrooms, over 1.5m away. When offered chickens as hosts, >20% of ticks fed. Over 98% of the human population lived in traditional 'Tembe' houses, and only 11% slept in raised beds. A total of 82% of households reported tick biting, which 85% believed was higher in the dry season. TBRF was perceived by the local population as second in importance to malaria as a cause of illness, and 86% knew it was transmitted by ticks. If TBRF infection was suspected, 84% said they would seek treatment at a health facility, while 9% would use one of nine known traditional medicines. Common methods used to reduce tick infestations in floors were plastering (96.5%), sweeping (80%) or sprinkling hot water (17%) or insecticides (20.7%).

In a randomised controlled trial of ITNs, acceptance of the intervention was high. Domestic tick infestation rates were reduced from 71.5% households at baseline to 10% and 41% in the treated and control groups respectively after 29 months ($p<0.001$). Infestation density changed from a mean of 4.91 ticks per infested house at baseline to 2.4 (treated) and 5.5 (control), a result that was also significant ($p<0.001$). Examination of ticks 12 months after the trial began found that only 13.8% of ticks in treated households were bloodfed as compared with 44.8% in the controls ($p<0.001$). At baseline, *Borrelia* infections were found in 2.8% of children under five years of age. PCR was twice as sensitive, showing *Borrelia* sp in 5.3% children. Genotyping identified *B. duttonii* and a new, unknown species. During the trial period, examination of 1799 blood samples (3 months post trial began) found significantly more *Borrelia* infections in the controls (17, 1.67%) than in the treated households (2, 0.26%), a difference that was significant ($p<0.05$). Examination of 6548 blood samples at the end of the trial found significantly more *Borrelia* infections in the controls (18, 0.54%) than in the treated households (2, 0.06%), a difference that was highly significant ($p<0.001$).These results demonstrated the efficacy of ITNs in reducing tick populations and transmission of *Borrelia*, despite the fact that 85% of control households were practising anti-tick activities by the end of the trial

The study has demonstrated the potential of ITNs for the control of domestic populations of *O. moubata s.l.* and for the prevention of TBRF. As such, a basis now exists for a new initiative on TBRF control in Tanzania and other affected countries.

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ASFV	African swine fever Virus
CI	Confidence interval
DNA	Deoxyribonucleic acid
Epi-Info	Epidemiological Information System
GIS	Geographical Information System
HIV	Human Immunodeficiency Virus
IRR	Incidence Relative Risk Ratio
ITMs	Insecticide Treated Materials
ITNs	Insecticide Treated Nets
IUCN	International Union for the Conservation of Nature and Natural Resources
JH	Jarisch-Herxheimer Reaction
KAP	Knowledge, Attitude and Practices
LBRF	Louse Borne Relapsing Fever
NGOs	Non Governmental Organisations
NIMR	National Institute for Medical Research
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PPF	Procaine Penicillin Fortified
QBC	Quantitative Buffy Coat
RBC	Red Blood Cells
RCT	Randomised Controlled Trial
RH	Relative Humidity
RR	Relative Risk Ratio
SMITN	Social Marketing Insecticide Treated Nets in Tanzania
SEM	Standard Errors of Mean
TBRF	Tick-Borne Relapsing Fever
WHO	World Health Organisation

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

Tick-Borne Relapsing Fever (TBRF) is a vector-borne disease of humans caused by bacteria of the genus *Borrelia* and transmitted by soft ticks (Argasidae) of the genus *Ornithodoros*. Transmission occurs through tick-bite (saliva) and also sometimes by contamination of the bite wound with tick-coxal fluid.

TBRF caused by *B. duttonii* is an endemic disease in Tanzania, and although possibly the third most important vector-borne disease in the country (after malaria and lymphatic filariasis), it does not feature in the Tanzania Ministry of Health Standard Treatment Guidelines. The National Health Management Information System (HMIS) does not have a separate category for this disease and so it is difficult to ascertain the actual number of cases. A major problem is that routine diagnosis of TBRF by microscopy and or symptomatic presentations is very difficult and TBRF is confused with malaria, and many TBRF infections in malaria endemic areas are possibly misdiagnosed and treated with antimalarials.

Like malaria, TBRF mostly affects pregnant women and children as this group has less immunity to infections. Several studies in Tanzania have shown that TBRF incidence can be as high as 38% in children under 1 year of age and 16% in 1-5 year olds (Barclay & Cutler, 1990); *Borrelia* prevalence rates of 7.5% can occur in pregnant women (Mushi, 1996) and 5.3% in under 5 year olds (Kisinza *et al.*, 2003). The risk of pregnancy interruption can be as high as 30% (Melkert, 1991). These figures call for mechanisms of early diagnosis, treatment and appropriate and sustainable community-based interventions to prevent transmission.

Following the introduction of insecticide treated nets (ITNs) for the control of malaria transmission in central Tanzania in 1996; it was observed that TBRF cases dropped by approximately 67% (Hospital records: *unpublished*). Yet there have been no studies conducted anywhere to evaluate the efficacy of ITNs in the control of TBRF transmission. The objective of this study therefore was to investigate aspects of natural history of *Ornithodoros moubata s.l.* in relation to TBRF transmission and determine the efficacy of insecticide treated bed nets for the reduction or elimination of both domestic tick infestations and transmission of TBRF.

1.2 LITERATURE REVIEW

1.2.1 Tick-Borne Relapsing Fever

Definition of TBRF

Tick-Borne Relapsing Fever (TBRF) was first recognized to be a tick-transmitted disease in 1905, when Dutton and Todd demonstrated spirochaetes in *Ornithodoros moubata s.l.* ticks in West Africa (Dutton, 1905; Raoult & Roux, 1999). Subsequently, it has been shown that TBRF is caused by at least 13 *Borrelia* species that are transmitted worldwide to people by soft ticks of the genus *Ornithodoros* (Sonenshine, 1993). Recently, more new *Borrelia* species have been identified and isolated using PCR in parts of the world such as Spain (Anda *et al.,* 1996) and Tanzania (Kisinza *et al.,* 2003). The disease is characterised by episodes of febrile illness separated by afebrile periods, giving rise to its name.

Distribution of TBRF

TBRF is widely distributed throughout the Eastern and Western Hemispheres (Burgdorfer, 1969; WHO, 1989; Estrada-Pe & Jongjean, 1999). In the Old World, TBRF is endemic in east, central and southern Africa, the Mediterranean region (North and West Africa) extending eastwards through Cyprus, Israel, Iran, Central Asia, Kashmir (India) to western China. In the New World, TBRF occurs in central and western USA, central and South America extending southwards to northern Argentina.

Several authors have suggested that the rapid environmental changes as a result human population increases have resulted in expansion of tick-borne disease (Walton, 1950; Hoogstraal, 1982 & Lane, 1994). However, there are few studies or surveillance programmes on the epidemiology and control of many tick-borne diseases including

tick-borne relapsing fever, which results in underestimation of the disease burden (Kitchen, 1972; Hoogstraal, 1981; Silayo, 2000; Lopez-Vedez, 2005).

Importance/ pathology

TBRF is an important cause of maternal and child morbidity and mortality in disease-endemic countries. Pregnant women and children under five years of age are most susceptible (Goubau *et al.,* 1983; Jongejan *et al.,* 1997). TBRF is also more serious in newcomers to an endemic area than in the indigenous people (*e.g.* neurological complications are far more common in visitors than in the local population), indicating that some level of immunity probably exists in persons under continuous challenge (Walton, 1962). Neurological complications are not uncommon appearing at the end of the first bout of fever or during relapses, and can result in ophthalmoplegia or deafness (Cook, 1996). Acute adult complications include jaundice, hepatomegaly, pneumonia and mental confusion (Dennis, 1998).

Epidemiology of TBRF

Several reports (Geigy & Mooser, 1955; Walton, 1962 & 1964; Burgdorfer, 1969) have described the epidemiology of Tick-Borne Relapsing Fever. TBRF is a zoonotic disease in all cases, with the exception of *B. duttonii* in Africa, where humans are believed to be the only known host. However, the recent study[1] has shown that domestic chickens and pigs are also potential reservoirs of *B. duttonii.* The reservoir animals are usually rodents, though certain insectivores are also reservoir hosts for *B. crocidurae* (Felsenfeld, 1965 & Trape *et al.,* 1991). The diversity of hosts infected with any one species of relapsing fever *Borrelia* is low compared with the Lyme disease agent, *B. burgdorferi*, probably a result of the more host-specific feeding preferences of the tick

[1] *PJ McCall, Jen CC Hume, Kefentse Motshegwa, Patricia Pignatell, Alison Talbert & William Kisinza* : "An animal reservoir of *Borrelia duttonii* in a TBRF endemic area of East Africa"(The Lancet: Submitted) , but as it post dates this thesis, it has not been incorporated in the literature review.

vectors rather than host specificity of the *Borrelia* (Felsenfeld, 1965). As *Ornithodoros* sp life cycles are spent in close association with the microhabitats where their hosts nest or rest (rather like fleas, Siphonaptera), they are unlikely to come into contact with any species other than their normal host (Walton, 1962). In many parts of the world (but not typically so in Africa) humans typically become infected when they come into contact with ticks, either by camping in wilderness areas, by sleeping in remote rodent-infested houses or caves or if the rodent hosts disappear due an epizootic disease (such as plague) and the ticks are driven to search for new hosts (Zumpt, 1959; Parola & Raoult, 2001).

Due to the sporadic nature of most TBRFs, reliable estimates for the prevalence or incidence of disease worldwide are rare. Tick-borne relapsing fever cases are typically isolated, often in small family clusters and rarely involving more than a few persons. Most patients are unaware of the tick bite and, if a history of camping or wilderness activity is not suspected, it can easily be misdiagnosed. The disease is sustained in endemic areas by both tick and rodent reservoir hosts, but ticks appear to be the main reservoirs (Walton, 1962, Hoogstraal, 1981; Younis, 1995).

In East and Central Africa, the situation is different. Here, *B. duttonii* persists as a low-prevalence disease in humans in communities within endemic areas. Transmission occurs in traditional houses, where the vectors, ticks species of the *O. moubata* complex, live within the home in cracks and depressions in the loose earth floors and walls (Walton, 1964; Geigy, 1968). Wide variations in feeding behaviour are seen within the *O. moubata* complex, influencing tick vectorial capacity (Walton, 1957). Prevalence of TBRF is higher where a human feeding habit predominates and where certain types of local housing promote the proliferation of indoor tick populations. Ticks probably colonize a new house by being carried unknowingly in the possessions of travellers moving between homes. They are nocturnal and emerge a few hours after dark to feed upon the sleeping occupants (Walton, 1962, Geigy, 1968). In Tanzania, house infestation rates as high as 88% have been reported (Talbert *et al.,* 1998), and biting rates can be so high that the occupants are driven to sleeping outdoors (*Author's*

personal observation). Ticks may then be found outside the house beneath these sleeping places (McCall, *personal communication*).

Clinical Characteristics of TBRF

The incubation period of TBRF varies from 2 to 18 days, with an acute onset of high fever with chills, headache, myalgia, arthralgia, and coughing (Barbour, 1990; Sonenshine, 1993; Parola & Raoult, 2001). Haemorrhage (rarely severe), iritis or iridocyclitis, hepatomegaly, or splenomegaly may also occur (Southern and Sanford, 1969), and abdominal pain, nausea, vomiting, diarrhoea, and photophobia are common in cases of infection in Africa (Cook, 1996). Rashes may occur at the end of the first febrile episode, and neurological findings are frequent and may be severe, particularly in infections with *Borrelia turicatae* (North America) and *B. duttonii* (Africa) (Cadavid and Barbour, 1998). Jaundice occurs in 7% of patients, and the case-fatality rate is approximately 2%–5% (Southern and Sanford, 1969). In general, the primary episode lasts for 3 days and is followed by a second, shorter, milder episode 7 days later. Thereafter, there may be more than 1 episode at 4 - (Africa) to 7- (United States) day intervals, each lasting for about 2 days (Sonenshine, 1993). The resolution of the disease has been linked to antibody production against the different antigenic types presented by *Borrelia* in the successive relapses (Felsenfeld & Wolf, 1973; Kehl & Farmer, 1986; Barbour, 1990).

Antigenic variation in Borrelia spirocheates

After transmission to humans, the spirochaetes enter the skin and subcutaneous tissues without causing a lesion and invade both systemic and lymphatic circulation, where they multiply (Dennis, 1998). During fever, spirochaetes appear in the peripheral blood. Relapsing fever *Borrelia* undergo antigenic variation within the host (similar to that seen in African trypanosomes) giving rise to a succession of antigenic types that are in turn responsible for the series of relapses (Barbour, 1990).

The spirochaetes avoid the immune response of the infected animal or person through antigenic variation (Kehl *et al.,* 1986). Therefore, relapse is an immunological phenomenon resulting from the inherent capacity of *Borreliae* to undergo one or more antigenic variations during the course of the disease (Kehl, *et al.,* 1986; Barbour & Carter, 2000).

Studies of large groups of patients (Barbour, 1990; Kehl, 1986; Felsenfeld, *et al.,* 1973) have shown that there are usually four to seven febrile attacks and that the interval between attacks is four to seven days. The first attack is typically the most severe by criteria of height and duration of fever. Succeeding attacks are usually milder. The numbers of *Borrelia* circulating in the blood are approximately 10-fold lower in the relapse peaks than during the initial attack. Antigenic variation poses a challenge in the development of vaccines against TBRF.

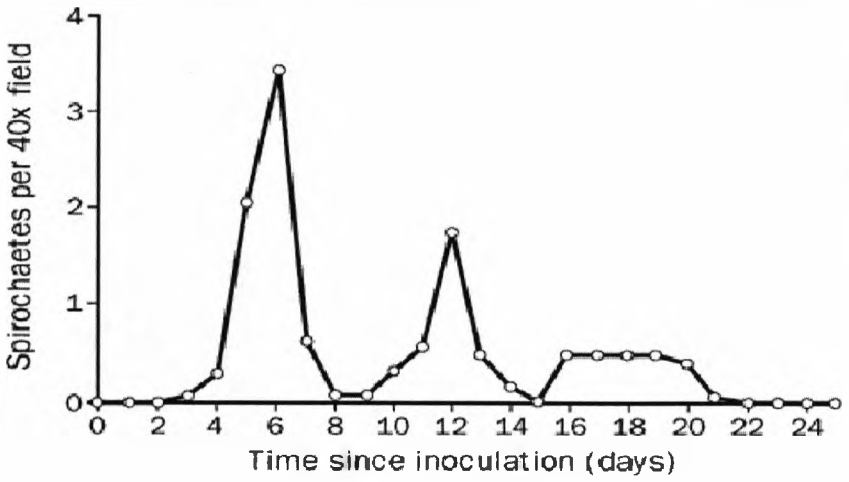


Figure 1.2.1(a): Cycle of spirochaetaemia of *Borrelia spp* .infection in mice (Kehl *et al.,* 1986)

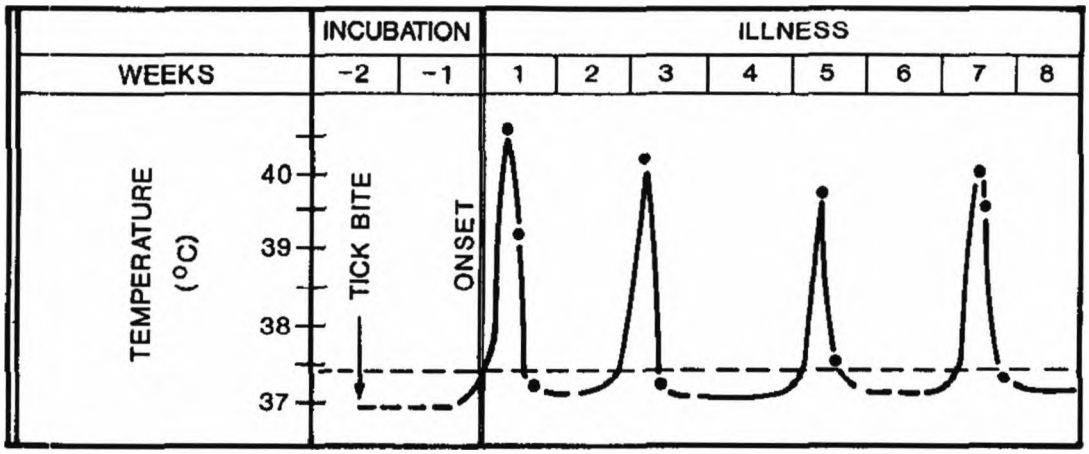


Figure 1.2.1(b): Body temperature of a patient with TBRF infected with *Borrelia hermsii* (Barbour, 1990)

Diagnosis of TBRF

Relapsing fever is difficult to distinguish from many other fevers before a remission-relapse cycle has occurred. It can be easily confused with malaria, typhus fevers, typhoid, meningococcal septicaemia/ meningitis, hepatitis, yellow fever, leptospirosis and other viral haemorrhagic fevers (Goubau, 1984; Schwan, *et al*, 1995; Gill & Beeching, 2005).

A diagnosis based on clinical signs only is difficult for TBRF, even more so when simultaneous occurrence of TBRF with malaria, typhoid fever is expected (Johnstone, 2003). TBRF diagnosis is usually made by demonstration of the spirochaetes in dark field preparations of fresh blood or stained thick or thin blood films taken during the febrile period; when fresh blood samples are scanned by dark field microscopy, spirochaetes can be readily detected under low power because of their locomotive characteristics: helical rotation and twisting movements in both directions (Barbour, *et al.,* 1986). TBRF spirochaetes have an affinity for acid dyes and stain readily with aniline dyes (Sciotto, 1983). Giemsa stain is used most often for staining spirochaetes in

thick and thin film preparations (Dutton & Todd, 1905; Gill & Beeching, 2005). Although not widely available, serologic testing for TBRF may be done using enzyme-linked immunosorbent assay (ELISA) tests (Voller, *et al.,* 1980; Schwan, *et al.,* 1992). More recently, an enzyme-linked immunosorbent assay that used the *GlpQ* gene product was demonstrated to be a useful diagnostic aid (Schwan *et al.,* 1996). Furthermore, this antigen is specific for the relapsing fever group Borreliae, thus distinguishing these from cases of Lyme borreliosis (Schwan *et al.,* 1996).

In poor rural communities in Africa (and also perhaps elsewhere) TBRF remains undiagnosed or is often misdiagnosed as malaria (Johnstone, 2003) or another non-specific fever and it is likely that prevalence of *B. duttonii* is much higher than that has been recorded (Kisinza *et al.,* 2003).

Detection of *Borrelia* by thick-blood films

Detection of spirochaetes in patient blood is considered valid evidence of tick-borne relapsing fever. The standard laboratory method of diagnosis of relapsing fever *Borrelia* is detection of spirochaetes in Giemsa-stained thick blood smears in peripheral blood of febrile patients (Schwan & Sanford, 1969). This test has a sensitivity of 70% when blood smears are examined by means of dark-field microscopy or when stained with Giemsa or Wright's stain (Southern & Sanford, 1969; Parola & Raoult, 2001). On the other hand, failure to detect spirochaetes microscopically is not considered conclusive, since spirochaetes may be extremely scarce and difficult to find, especially during late relapses (Kehl & Farmer, 1986; Cook, 1996).

Thick blood film is a concentration method and permits examination of a relatively large amount of blood on a small area of a microscope slide. It can routinely be applied to initial diagnosis because spirochaetaemia is often mild and tends to become milder with each succeeding relapse. At the time of fever, numerous spirochaetes can be found in the blood of the patient. During between fever periods, spirochaetes are not detectable in the blood (Kehl & Farmer, 1986; Cook, 1996).

The reduction in the numbers of circulating spirochaetes between the attack peak and the relapse peaks has not been explained. However, the lower number of spirochaetes circulating in the blood stream of a patient does not reduce the virulence of the *Borrelia* pathogens (Felsenfeld, *et al.,* 1973; Kehl, 1986; Barbour, 1990).

Serological tests

Serological tests are the most available and simplest diagnostic tests available for the study of infectious diseases. Numerous serological tests have been described, including complement fixation, indirect hemagglutination, latex agglutination, ELISA, immunoperoxidase, immunoblot assays, and immunofluorescence, which is the reference method in most laboratories for bacterial tick-borne diseases. The major limitation of serological tests is the cross-reactivity that might be present between antigens of pathogens within the same genus and also in different genera (Parola and Raoult, 2001). For example, the Weil-Felix test, the first serological assay for rickettsioses, is based on the detection of antibodies to various *Proteus* species, which contain lipopolysaccharide antigens that cross-react with rickettsial antigens (Lascola and Raoult, 1997). Because of the cross-reactivity between pathogens, serological results need to be interpreted very carefully to prevent mistakes being made in the discovery and description of the epidemiology of tick-borne bacterial diseases.

Serodiagnosis of TBRF is very difficult and not reliable (Brown, *et al.,* 1999). No specific or standard procedure for Serodiagnosis of TBRF has been developed because of the complexity of the relapse phenomenon. It is difficult to produce specific antiserum since spirochaetes undergo one or several antigenic variations during the course of illness (Kehl, 1986; Barbour, 1990).

Detection of spirochaetes by Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a molecular technique for amplifying (creating multiple copies of) DNA without using a living organism (Fukunanga, *et al.,* 2001; Sparagano, 1999; Higgins 1995). PCR is commonly used in medical and biological research laboratories for a variety of tasks, such as the detection of hereditary diseases,

identification of genetic fingerprints, diagnosis of infectious diseases, cloning of genes, and paternity testing. PCR has very quickly become an essential tool for improving human health and human life. Medical research and clinical medicine benefit from PCR mainly in two areas: detection of infectious disease organisms, and detection of variations and mutations in genes, especially human genes. The method is especially useful for searching out disease organisms that are difficult or impossible to culture, such as many kinds of bacteria, fungi, and viruses, because it can generate analyzable quantities of the organism's genetic material for identification (Fukunanga, *et al.,* 2001; Sparagano, 1999; Higgins 1995).

Quantitative Buffy Coat (QBC) Test

The Quantitative Buffy Coat (QBC) parasite detection method is a sensitive and specific tool for the diagnosis of malaria parasites. It is also useful for the diagnoses of other haemoparasites, including *Trypanosoma, Babesia*, and *Leptospira* (Chatel *et al.,* (1999). The study conducted in West Africa in 1999 showed that QBC technique is also useful in the diagnosis of tick-borne relapsing fever (*B. crocidurae)* and it can be as much as 100-fold more sensitive than thick smears (Van Dam, *et al.,* 1999).

Detection of Borrelia spirochaetes in soft ticks

Detection of spirochaetes in ticks can be achieved by (1) direct microscopic examination of haemolymph, coxal fluids, and tissue preparations, (2) injection of tick triturates into susceptible animals, (3) transmission experiments and (4) PCR.

In hard ticks, which may have been without a blood meal for several months or years, the degree of spirochaetal infections in various tissues is generally low except for the central ganglion, which always remains heavily parasitized and in this case microscopic techniques can not readily detect spirochaetes from the tick-vectors (Burgdorfer, 1969).

The advent of molecular methods, such as sequence analysis of PCR products, has enabled the rapid detection and identification of tick-borne pathogens. Such techniques may be used to screen large numbers of ticks during surveys or single ticks that have

been collected from patients suspected of having tick-borne diseases. Identification strategies based on the DNA sequences of a number of genes have been described (Fukunaga, 2001; Sparagano, 1999).

A nested-PCR based on the flagellin gene, has been developed and has been shown to be suitable for both detection and classification of the *Borrelia* genus (Fukunaga *et al.*, 2001) and was used to detect *Borrelia* infections from ticks (Fukunaga *et al.*, 2001 & Mitani *et al.*, 2004)and humans (Kisinza *et al.*, 2003) in Tanzania.

Treatment of TBRF

The mainstay of treatment is antibiotics-primarily tetracycline, doxycycline or penicillin (Butler, 1985). In the treatment of tick-borne fever, procaine penicillin fortified (PPF) is the drug of choice (Butler, 1985; Cook, 1996). Children under five years old are treated with procaine penicillin fortified with iron, 120 mg intramuscular injection daily for 5 days. The severe Jarisch-Herxheimer (JH) reaction is less common than with Louse-borne relapsing fever (LBRF), but can occur. The JH reaction to LBRF produces apprehension, diaphoresis (excessive sweating), fever, tachycardia, and tachypnoea (Sonenshine, 1990; Jongejan *et al.*, 1997).

1.2.2 Vectors of TBRF

Ticks

Ticks are among the most important vectors of diseases affecting both humans and animals (Sonenshine, 1991). Ticks are obligate haematophagous arachnids that parasitize every class of vertebrates in almost every region of the world (Parola and Raoult, 2001). Approximately 850 tick species have been described worldwide (Furman and Loomis, 1984) and belong to two major families, the Ixodidae (hard ticks) comprising 650 species, and the Argasidae (soft ticks) comprising 150 species. Ticks transmit the widest variety of pathogens, including bacteria, rickettsiae, protozoa, and

viruses. Important tick-borne pathogens include Lyme disease, ehrlichiosis, babesiosis, Rocky Mountain spotted fever, tularaemia, and tick-borne relapsing fever.

Soft ticks (Argasidae)

Life cycle

The first life stage to emerge from the egg is a six-legged larva that takes blood meal from a host, and moults to the eight-legged first nymphal stage, often while still within the egg (Service, 2004). Oviposition occurs between 10 to 15 days after mating and hatching of eggs takes place between 8 to 12 days at a temperature of 30°C (Kettle, 1984; Service, 2004). The life stages of soft ticks are not readily distinguishable. Unlike hard ticks, many soft ticks go through multiple nymphal stages, gradually increasing in size until the final moult to the adult stage. Some soft ticks pass through up to seven nymphal moults before they become adults (Varma 1962; Geigy 1968).

The eggs are laid in places where the adult ticks rest, such as cracks and crevices in the walls and floors of houses and in furniture (Kettle, 1984). A blood-meal is essential for egg production. Eggs usually hatch after 1-2 weeks but can remain viable for many months under adverse conditions (Service, 2004). The time to completion of the entire life cycle is generally much longer than that of hard ticks, often lasting over several years. The duration of the life cycle, from egg hatching to adult depends on the species of ticks, temperature and the availability of food-meals, but in argasids is typically about 6 – 12 months. Adult ticks can live for many years, up to 12 – 20 years in the laboratory. Additionally, many soft ticks have a resistance to starvation, and can survive up to 12 years without a blood meal (Furman and Loomis 1984; Kettle, 1984; Service, 2004).

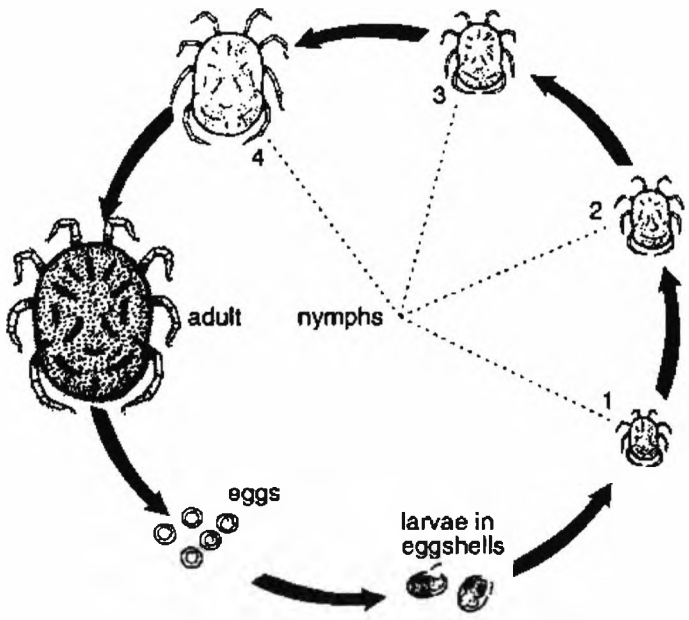


Figure 1.2.2(a): Life cycle of *Ornithodoros moubata s.l.* showing larvae retained in eggshells and nymphal stages (Service, 2004)

Morphological appearance

Sonenshine (1991 & 1993) described the morphology of soft ticks. The adults are flat and oval in outline and have tough, leathery, wrinkled bodies. The mouthparts of soft ticks are not visible from above. Ventrally, there are only three external visible mouth components: the two outside jointed parts are the highly mobile palps; between these are paired chelicerae, which protect the centre rod-shaped tubular feeding structure, the hypostome. The palps move laterally while the tick is feeding and do not enter the skin of the host. The rough hypostome has many beak/ tooth-like projections on it. This is the structure which plunges into the host's skin while feeding. The backward directed projections facilitate adherence to the host during feeding (Sonenshine, 1993).

Feeding behaviour

Soft ticks may feed several times during each life stage and all nymphal stages, adults and both sexes all actively search for hosts from which to take blood-meals. After

feeding, which lasts about 30 minutes; they drop to the ground. Females lay multiple small batches of eggs between blood meals throughout their lives. Most species can survive for more than a year between blood-meals, and some for more than 10 years (Geigy 1968; Service, 2004).

Ticks are attracted by the emanations and warmth of the host's body as well as attractants such as carbon dioxide, which serve as stimuli for host seeking behaviour (Burgdorfer, 1969; Cupp, 1991; Estrada-Pena & Jongejan, 1999; Parola, 2001). The feeding behaviour of soft ticks is comparable to that of fleas or bedbugs as they usually stay in the proximity of their hosts. Soft ticks are adapted for feeding rapidly and leaving promptly, and are rarely seen on the host. Hosts may not be aware of the feeding ticks. While feeding, the soft tick expands to accommodate the large volume of blood to be ingested, which may be anywhere from 5-10 times their unfed body weight (Sonenshine, 1991).

Natural history

Several studies (Walton, 1957; Varma, 1962; Galun & Sternberg, 1978; Geigy, 1968; Parola, 2001) described the biology of soft ticks. Freshly fed female ticks mate, go underground, and soon afterward lay multiple small batches of about 80 eggs measuring of about 0.6mm in diameter. At a temperature of more than 25°C the larvae hatch within 10 to 12 days (Service, 2004).

Habitats and geographical distribution of TBRF vectors in the genus *Ornithodoros*

The widely distributed argasid genus *Ornithodoros* has several representatives involved in the parasitism of humans. In summary, a total of 22 species of 87 *Ornithodoros* species have been reported on humans, and 12 species are found frequently (Estrada-Pen & Jongejan, 1999).

Since most *Ornithodoros* ticks are typically nidiculous parasites, humans are particularly involved in the cycle of transmission when they intrude into the nest environment. Thus, relapsing fever is most often zoonotic in character with a variety of

nesting or burrowing inhabiting mammals, especially rodents, acting as reservoirs (Sonenshine, 1993). In such cases the epidemiology is characterized by isolated outbreaks in scattered localities inside endemic areas (Burgdorfer, 1986).

Borrelia spirochaetes persist in the argasid vectors for many years, probably for their entire life. Thus, the longevity and cryptic habits of the *Ornithodoros* ticks perpetuate this zoonotic form in endemic areas (Sonenshine, 1993).

The main vector of TBRF in North America, *O. hermsi, is* found in cavities of dead trees, log cabins and human dwellings, and usually feeds on tree squirrels. People become infected with *Borrelia hermsi* when rodents are driven out during rodent-trapping activities (Sonenshine, 1993).

Borrelia parkeri, transmitted by the bite of *Ornithodoros parkeri* (Western United States, California), seldom if ever infests man, although the tick vector feeds on the same hosts and occurs in the same geographical area as *O. hermsi*, *Ornithodoros talaje* is distributed throughout the western states of the USA, Mexico, Venezuela, Uruguay, Brazil, Panama, Ecuador, Chile and Argentina (Estrada-Peña & Jongejan, 1999). *O. talaje* can transmit *Borrelia talaje* to man in some parts of central and South America, although adults of this tick are seldom observed in dwellings and are not parasites of man.

Ornithodoros turicata inhabits the south-western part of the USA and Central America, and is a vector for *Borrelia turicatae* (Burgdorfer, 1980). Few human infestations have been recognized in the USA, but in Oklahoma, where *O. turicata* infests homesteads and sheds, entire families were developed relapsing fever (Burgdorfer, 1980). Typical of *Ornithodoros* species, the bite of *O. turicata* is painless, but is usually followed by intense local irritation and swelling several hours later and subcutaneous nodules may persist for months (Cooley and Kohls, 1944).

Ornithodoros amblus has been occasionally reported on humans in Peru (Clifford *et al.*, 1980). *Ornithodoros rudis* is distributed throughout Panama, Paraguay, Colombia, Venezuela, Peru and Ecuador and is the primary vector of *Borrelia venezuelensis*, although clinical and epidemiological details are poorly documented (Hoogstraal, 1985).

Ornithodoros rostratus has been implicated in human parasitism in South America, where it is well-known for its painful bite, which often becomes pruritic and secondarily infected. *O. rostratus* is able to transmit *R. rickettsii* (Hoogstraal, 1985).

In the Caucasus and Iraq, *Ornithodoros asperus* inhabits rodent burrows, and transmits *Borrelia caucasica*, which can cause severe disease in humans, with numerous rapidly recurring relapses (Estrada-Peña & Jongejan, 1999). *Ornithodoros coniceps* has been reported on humans in France and Spain (Gil Collado, 1947; Keirans, 1984), usually as the result of sleeping in caves where rock pigeons nest. Human hosts develop local oedema and pain with chills lasting from a few hours up to three days (Hoogstraal *et al.*, 1979).

Ornithodoros erraticus occurs in Spain and parts of north and West Africa. This species transmits *Borrelia crocidurae* (North and East Africa) and *Borrelia hispanica* (Tunisia, Algeria, Morocco, Spain and Portugal). Reports of human cases of relapsing fever due to *B. crocidurae* have become rare in North-east Africa, where the disease has declined since the 1940s (Trape *et al.,* 1996). However, Trape *et al.* (1991) and Vial *et al.* (2006) reported wide distribution of *B. crocidurae* in Senegal, where relapsing fever appears to be a major cause of morbidity in rural areas.

Anda *et al.* (1996) reported the isolation of a new *Borrelia* species from patients with relapsing fever and from *Ornithodoros spp.* in southern Spain. This pathogen is closely related to other tick-borne relapsing fever spirochaetes in Europe and Africa, and causes a disease with serological similarities to Lyme disease. *Ornithodoros maritimus* is commonly found on sea birds, but has also been reported on humans in France (Chastel

et al., 1984). It transmits Soldado virus, a public health problem in urban environments where sea gulls feed. *Ornithodoros lahorensis* is a common ectoparasite of man found in most parts of the former Soviet Union and can be infected experimentally with *Rickettsia sibirica* (Sidorov and Kokorin, 1980).

Ornithodoros tartakovskyi, distributed throughout most of Central Asia, may be infected with *Borrelia latyschevi* the agent of Central Asia relapsing fever, which can cause a mild human illness (Sidorov and Kokorin, 1980). *Ornithodoros tholozani* commonly lives in man-made shelters, caves, rocky overhangs and other localities where livestock is housed (Arthur, 1962). The species is distributed from China to eastern Libya, and transmits *Borrelia persica*, the agent that causes Persian relapsing fever, resulting in a severe and sometimes fatal human disease (Estrada-Peña & Jongejan, 1999).

Ornithodoros capensis is a cosmopolitan tick of seabirds (Keirans *et al.*, 1992), which has also been reported on humans. Visitors to penguin breeding sites in caves and on barren coasts have been attacked by nymphs and adults of *Ornithodoros spheniscus* with subsequent pruritic, slowly-healing blisters (Hoogstraal *et al.*, 1985).

Although hepatitis virus does not multiply in soft ticks, mechanical transmission from ticks to man may occur by crushing infected ticks, through a bite, or by contamination with coxal fluid when scratching tick bite lesions (Jupp *et al.*, 1987). Joubert *et al.* (1985) detected this virus in *O. moubata* collected from the north-east strip of Namibia and suggested that mechanical transmission may be responsible for the high prevalence rate of hepatitis virus infection in humans in this area.

Ornithodoros muesebecki has been collected from humans in Arabia (Hoogstraal, 1982), where its bite causes blisters, pruritus and fever. It has not been determined whether salivary toxins or pathogens are involved. *Ornithodoros savignyi* is found in human habitations in India, Africa and some parts of Asia (Keirans, 1984; Hoogstraal, 1985) and causes intense local irritation in man (Hoogstraal, 1956). Moreover, in dry

areas of Africa and Asia *O. savignyi* commonly attacks humans resting under shady trees and around wells where animals gather.

Table: 1.2.2: Geographical distribution of important *Ornithodoros* species

***Ornithodoros* species**	**Species of *Borrelia***	**Distribution**
O. moubata	*B. duttonii*	East Africa and Southern Africa
O. parkeri	*B. parkeri*	Central and Western USA, Mexico
O. asperus(=O. verrucosus)	*B. caucasica*	Caucasus, Iraq
O. coriaceus	*B. coraciae*	Pacific coast of USA into Mexico
O. erraticus (= O. sonrai)	*B. crocidurae*	West and East Africa and South Europe
O. erraticus	*B. hispanica*	Spain, Portugal, North Africa
O. hermsi	*B. hermsi*	Central and Western USA, Mexico
O. tartakovskyi	*B. latyschevi*	Central Asia, Iran
O. turicata	*B. turicatae*	Southwestern USA, Central America

*Source: (*Estrada-Peña & Jongejan, 1999)

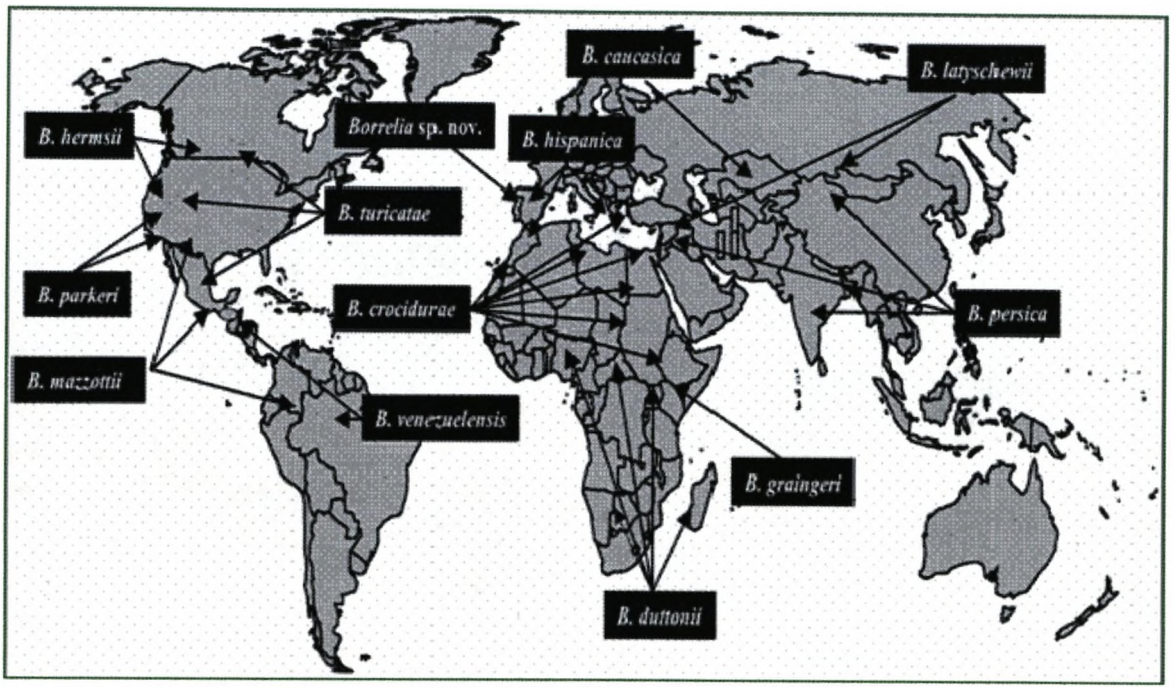


Figure 1.2.2(b): Geographical distribution of tick-borne relapsing fever *Borreliae* (Source: Parola & Raoult, 2001)

Development of *Borrelia* in the vector tick

Ingested spirochaetes multiply in the mid-gut of the tick, penetrate the gut wall and disperse to the tick's organs and can be found in the salivary glands, ovaries and coxal glands after about 3 days Kettle, 1984; Service, 2004). On subsequent feeding, the host can be infected by the infected saliva or through infected coxal fluid (released by the tick during feeding) contaminating the bite wound (Walton, 1957; Geigy & Aeschlimann, 1964; Sonenshine, 1993).

Several reports (Aeschlimann, 1958; Geigy, 1968; Van der Merwe, 1968; Plowright *et al.,* 1970a; Burgdorfer & Brinton 1975) described transmission of *Borrelia spp.* to *Ornithodoros spp.* There are two major routes of *Borrelia* transmission: (1) feeding on infected hosts and (2) transovarial transmission (the *Borrelia spp.* pass from the female infected ticks to eggs). Ticks can inherit the infection from their mother and the infection is retained by individual ticks through each moult (transstadial transmission). Once ticks are infected, they remain infected for life (Sonenshine, 1993). This occurs at

a high rate permitting maintenance of the parasite in the tick population for generations, without the need for an individual tick to feed on an infected vertebrate at any stage in its life. This, in combination with the ability of the ticks to starve for long periods, enables some natural TBRF foci to survive for long periods in the absence of man (Felsenfeld, 1965; Burgdorfer, 1975; Galun, 1978).

Transovarial transmission is common in vector-pathogen relationships and it is a mechanism used by many both bacterial spirochaetes and arboviruses to ensure survival during adverse conditions (Burgdorfer & Brinton 1975). Examples of this are the mosquito-borne bunyaviruses of the California serogroup, which use transovarial transmission as an over wintering mechanism (LeDuc, 1989). Viruses from this group are therefore able to survive in temperate or arctic regions where climatic conditions preclude year-round transmission. Similarly, as tick vectors can survive for considerable lengths of time without a blood meal, transovarial transmission provides a mechanism by which *Borrelia* spirochaetes can survive within the tick population in the absence of re-infection from a viraemic vertebrate host (Burgdorfer & Brinton 1975).

There is little information about the consequences of bacterial spirochaetes infection on the host ticks themselves, but lowered fertility and high mortality have been reported in ticks infected with *Rickettsia rickettsii* (Niebylski, *et al.,* 1999).

1.2.3 African Swine Fever Virus (ASFV)

African Swine Fever Virus (ASFV) is a serious arboviral disease of pigs occurring in Africa caused by arbovirus which is transmitted to pigs by *Ornithodoros moubata* species. Also a number of North and Central American and Caribbean species of *Ornithodoros* have been shown to be potential vectors of ASFV (Kleiboeker and Scoles, 2001). ASFV causes a lethal haemorrhagic disease in domestic pigs. ASFV is enzootic in sub-Saharan Africa and is maintained in a sylvatic cycle by infecting both wild warthogs and *O. moubata s.l.* (Taylor & Colquhoun, 1977). The pathogenesis of ASFV in *Ornithodoros* ticks has been reported to be characterized by a low infectious

dose, lifelong infection, and efficient transmission to both pigs and ticks (Penrith, 1997).

ASFV pathogenesis in warthogs is characterized by an unapparent infection with transient, low viraemic titers. Thus the *Ornithodoros* ticks probably constitute the most important natural host of ASFV, although both the mammalian and tick hosts are probably required for the maintenance of ASFV in the sylvatic cycle (Rennie, *et al.*, 2001). The disease can cause up to 10% mortality to pigs in 7 – 10 days after exposure. In Africa, pigs are at high risk of ASF as the village pigs just roam and the owners do not practice isolation procedures when the pigs are infected. Once the disease is introduced into a herd, it spreads by direct and indirect contact with secretions and excretions from infected pigs (Stone & Hess, 1973). ASFV is a tick virus and the pig is an accidental host (Plowright, 1977). ASFV replicates in the tick and that there is transstadial, transovarial, and sexual transmission in *Ornithodoros* ticks (Hess, 1987). The African swine fever in wild pigs in Africa is believed to cycle between soft ticks living in warthog burrows and newborn warthogs (Thompson *et al.*, 1980) (see figure 1.2.3).

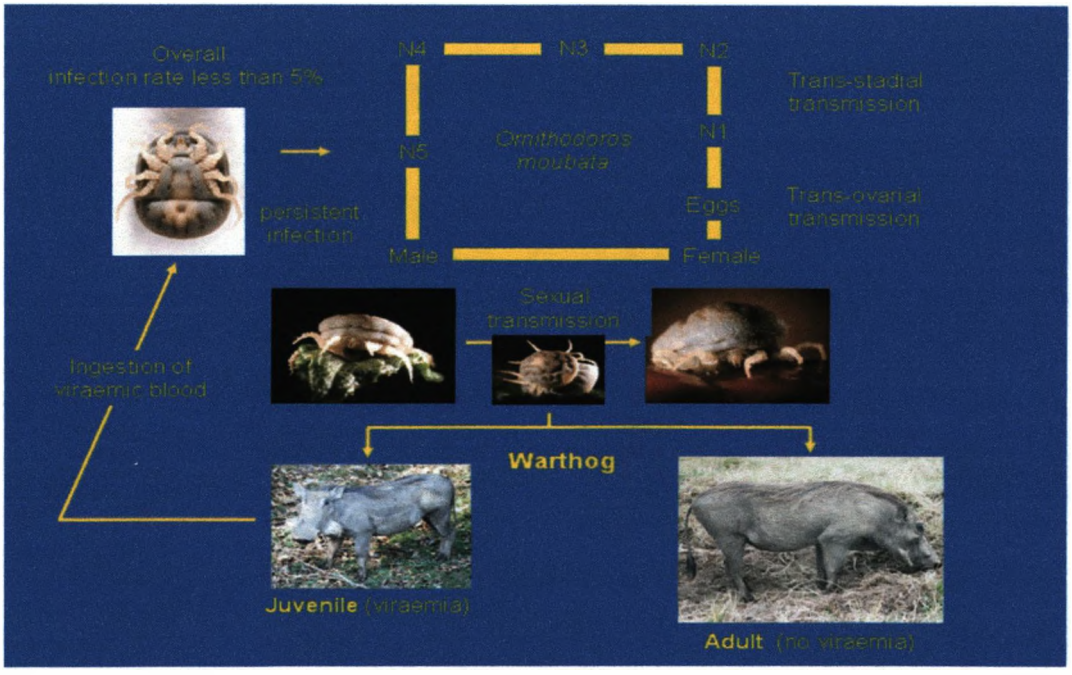


Figure 1.2.3: ASFV transmission cycle (Thompson *et al.*, 1980)

1.2.4 The *Ornithodoros moubata* complex

Since Walton's work on the *Ornithodoros moubata* complex in East Africa during the 1940s and 1950s, few studies of any kind have been made on the vectors of TBRF in Africa. In particular, the taxonomy of this group has been ignored. *Ornithodoros moubata sensu lato (s.l)* is a complex of species or subspecies, each differing in its ecological requirements, feeding behaviour (host preferences) and vectorial capacity (Walton, 1962, 1964).

Based on morphology, Walton (1962, 1964) classified *O. moubata* as a complex of four species or subspecies. Two species, *O. compactus* and *O. apertus* were thought to feed on tortoises in South Africa and porcupines at Lake Naivasha respectively. Walton erected a third and fourth species, *O. moubata* and *O. porcinus*. *O. moubata* was separated further into two races ('wild' zoophagic or 'domestic' anthropophagic), depending on whether they infected human dwellings or warthog and porcupine burrows (Walton, 1962, 1964). *O. porcinus* was considered to have a very wide distribution, occurring in both human dwellings and warthog burrows from central Kenya to Mid-Mozambique. Based upon feeding habits, this genus was separated further into races or subspecies called *O. porcinus domesticus*, *O. porcinus porcinus* and *O. porcinus avivora*. *O. porcinus domestica*, *O. p. porcinus* and *O. moubata* domestic form were considered to be good vectors of *B. duttonii* (Walton, 1979).

Clearly, *O. moubata* is a complex, and different species may differ in their ability to transmit different *Borrelia spp.* and other pathogens. But how the *Ornithodoros moubata* species complex is structured is unknown. Walton's classification of *O. moubata* into four major species varying in their distribution and host preference has been disputed (Van der Merwe, (1968) but based as it is on comprehensive collection of material and observation of tick natural history from a wide geographical area; it remains the most thorough investigation.

Since then the complex has not been investigated further. The number of species or subspecies within the *O. moubata* complex remains unresolved. For the purpose of this study the ticks within the *O. moubata* complex will be referred to simply as *O. moubata sensu lato (s.l.)*.

1.2.5 TBRF in Africa, with particular reference to Tanzania

Several studies and reviews (Ordman, 1957; Teesdale, 1965; Burgdorfer, 1969; Goubau, 1984; Trape, 1991; Dumler, 1995; Camicas *et al.,* 1998; Parola, 2001) have described TBRF in Africa. East African TBRF is probably distributed across a wide area including Ethiopia, Somalia, Kenya, Uganda, Tanzania, Rwanda, Burundi, Central and Eastern Congo, Malawi, Zambia, Zimbabwe, South Africa and Madagascar. The main pathogenic agent is *Borrelia duttonii* whose natural vector is *O. moubata* species, although a second, as unnamed yet species has also now been found (Kisinza *et al.,* 2003) appended.

In West Africa, TBRF is caused by *Borrelia crocidurae* and occurs in the drier Sahel and Saharan regions where the vector tick *Alectorobius sonrai* is distributed (Trape *et al.,* 1991, 1996 & 1997). TBRF is a major cause of high morbidity for the children under five years old in West Africa where the TBRF annual incidence rates ranged between 1.6% and 4.2%. TBRF incidence in the region reflects the close association with the vector ticks and with the rodent reservoirs since burrows are frequently found inside or near the houses (Trape, *et al.*, 1997).

Trape *et al.* (1997) showed that the spread of TBRF in West Africa has a close relationship with drought that allows the vector tick *Alectorobius sonrai* to colonize new areas. Therefore with the current global warming trend and its associated climatic changes expected to affect a wide range of ecological processes, there may be serious consequences for TBRF (Cutler, 2006).

A study conducted in the Democratic Republic of Congo in 1997 found that the incidence of *B. duttonii* relapsing fever among pregnant women in the maternity wards was 6.4% and the prevalence of the disease in the outpatients ranged between 4.3% and 7.4%, often leading to maternal death or to spontaneous abortion (Williams *et al.*, 1997). TBRF was also found to be endemic in the rural settings of west Rwanda (Malatre, *et al., 1991*).

Several studies described TBRF related pregnancy interruptions (Goubau and Munyengeyo, 1983; Melker, 1988 & 1991; Barclay and Cutler 1990, Jongejan *et al.*, 1997). During pregnancy, *Borrelia* spp. infection is particularly intense and pregnant women have higher spirocheate burdens, leading to high fever with a 30% risk of pregnancy loss and a perinatal mortality rate of 15% (Melkert, 1988). Barclay & Cutler (1990) found that the incidence of TBRF in Mvumi hospital clinic was 38% in children under 1 year of age and 16% in under 5 year-olds. The *B. duttonii* prevalence rate in Makang'wa village was reported at 7.5% in pregnant women and 3% in children under five years old at the local mother & child clinic (Mushi, 1996). Throughout East Africa, the mortality rate from TBRF in different locations is believed to range from 0-8%, with pregnant women and children most susceptible (Goubau, 1984).

Jongejan and colleagues (1997) published a study of 257 women with TBRF treated at Ndala Hospital in Tabora region West Tanzania between 1985 and 1995. The case fatality rate was 1.5% in pregnant women and 1.7% in non-pregnant women. Melkert (1988) reported a case fatality rate (CFR) of 3.7% in pregnant women with TBRF seen at Sengerema Hospital in Mwanza region and a report from Rwamagana Hospital in Rwanda gave an even higher CFR of 8.5% (Goubau and Munyangeyo (1983).

Jongejan (1997) also found that TBRF had a profound effect on pregnancy outcome with perinatal mortality of 436 per 1,000 births. The total loss of pregnancies including abortions was 475 per 1000 (47.5%), and the relapse rate was 3.6%, compared to 1.7% in non-pregnant women. The risk of delivery during the attack was positively correlated with increasing density of the spirochaetemiae.

Incidence and prevalence studies of *B. duttonii* infection have been carried out on humans in Dodoma Rural District. One series by Mushi (1996) in Makang'wa village found slide positive rates of *Borrelia* in 3.2% of adults (12/380), 2% of schoolchildren (2/100), 3% of children under 5 years old (3/100) and 7.5% of pregnant women (3/40). Recent studies in central Tanzania (Kisinza, *et al.,* 2003) found that 4% of healthy afebrile children (13/307) and 11% of febrile children (6/54) were positive for *Borrelia.*

1.2.6 Control of TBRF in Africa

Several attempts have been made to the control domestic tick-infestations and TBRF transmission in Africa. Teesdale (1965) and Walton (1964) maintained that, in houses infested with *B. duttonii,* construction of solid floors and walls with concrete or mortar severely reduced tick breeding and resting sites and eliminated infestations in Meru district in Kenya and in South African states. However, many subsistence African families in endemic TBRF areas can not afford the costs of cement and other building materials.

Talbert *et al.* (1998) showed that spraying homes with insecticide is an effective means of tick control. However, indoor residual spraying (IRS) has a number of drawbacks as discussed by Curtis (1990; Guyatt *et al.,* 2002a) regarding the control malaria, including the excito-repellent effect of insecticide, difficulty of access by spray teams to houses and unsightliness and smell of spray deposits. This method is also very expensive to sustain as it involves paying trained spray-teams which must be transported to cover all TBRF endemic areas in the country. Walton (1964) reported in Kenya that sleeping on raised beds can reduce tick biting and probably domestic tick infestation, however this is not effective as ticks can climbs on bed steads and majority of people in rural African settings sleep on floors using matting blankets of animal hides making them easy targets for nocturnal *Ornithodoros* ticks living in earth floors.

1.2.7 The use of insecticide-treated materials for vector control

ITNs have proven to be effective in reducing malaria mortality and morbidity both in Africa (Lengeler *et al.,* 1996) and Latin America (Kroeger *et al.,* 1995). That insecticide-treated nets protect against malaria are now beyond any doubt, and the promotion of their usage forms the basis of malaria control in the majority of endemic regions worldwide. Large studies in The Gambia, Ghana, Tanzania and Kenya have documented a 20% reduction in overall child mortality as a result of ITN use (Lengeler *et al.,* 1996).

Collectively, these studies reveal the potential of ITNs as an important public-health intervention in the control of malaria. Similarly, insecticide treated nets have also been shown to be effective in vector control of leshimaniasis in S. America (Kroeger *et al.,* 2002). ITNs typically used against flying vectors, they may also prevent biting by crawling vectors, like the Triatomine bug vectors of Chaga's disease in South America (Kroeger, et al, 2003). ITNs are distributed widely and their use against malaria is heavily promoted across Africa, particularly in Tanzania. ITNs are extremely effective in the prevention of transmission of malaria and lymphatic filariasis (WHO, 1995).

1.2.8 ITNs in Tanzania

Over the last twenty years, much work has taken place on ITNs in Tanzania. Various research organizations, donor agencies, non-governmental organizations (NGOs), the private sector and government agencies have been involved in improving this tool and preparing large scale expansion (Magesa *et al.,* 2005). Over 90% of Africa's net manufacturing capacity is based in Tanzania which means that the country has an unprecedented opportunity to reach even the most vulnerable groups with ITNs delivered through the commercial sector (Maxwell *et al.,* 2006).

An important development in Tanzania was the design and testing of insecticide home treatment kits (Miller *et al.,* 1999). Social Marketing Insecticide Treated Nets (SMITN)

and Kilombero Insecticide Treated Nets project in Tanzania (KINET) data suggest that affordability remains a significant obstacle to net use, especially for the poorest (Abdula, 2000). Recent data confirm a socio-economically stratified gradient in treated and untreated net ownership and re-treatment rates (Nathan *et al.,* 2004) although this gap is narrowing over time (Magesa *et al.,* 2005). Further studies on the issue of net re-treatment highlighted the difficulties associated with sustaining this behaviour (Winch *et al.,* 1997; Schellenberg *et al.,* 2002).

Ensuring availability of ITNs to all is essential. The Tanzania ITN voucher program targets subsidy to those who need it most (Tami *et al.,* 2005). Pregnant women (who are at risk of severe malaria) receive a voucher during antenatal visits and are then able to use the voucher as part payment for an ITN at the nearby retail or shifting market outlet. In this way, the commercial sector plays a central role in reaching vulnerable groups with ITNs. ITNs have been thought of mainly as personal protection, but there is increasing evidence that high coverage of ITNs within a community will reduce malaria transmission and other vector-borne diseases including TBRF because of the mass effect on vector survival (Gimnig *et al.,* 2003).

For nets to be maximally effective coverage must also be high, nets should be re-treated promptly (or long-lasting nets used) and individuals should properly deploy their nets each night. In Tanzania, the household coverage with ITNs has changed from around 37% in 2001 to over 50% in 2004 and household surveys have shown that children under five years are given priority to sleep under these nets (United Republic of Tanzania, National Census, 2002). As a result, Tanzania is now close to achieving the Abuja target of covering 60% of children under five and pregnant women with an insecticide treated net by the end of 2005.

One of the key constraints to the large-scale and sustainable use of ITNs is the need for regular insecticide re-treatment (every six months), and the fact that they lose efficacy after three washes. An ITN that is not re-treated rapidly becomes an untreated net, and is less effective (Guillet *et al.,* 2001). Unless a strategy for re-treatment is built into an

ITN distribution programme, the distribution will have no lasting value. Successful strategies for re-impregnation have, however, proved very difficult to identify. Re-treating nets is now easier than in the past: tablets or sachets are available to treat the nets, and they do not require any technical skill to use. The arrival of long-lasting impregnated nets will greatly improve treated-net coverage rates.

It has been suggested that ITNs should be viewed as a public good, like vaccines, and should be provided via the public sector with generous assistance from donors (Curtis *et al.,* 2003). Like vaccines, ITNs have both a personal protective effect to the individual user, as well as a community-wide effect because the occupied nets act like baited traps for mosquitoes (Magesa *et al.,* 1991). The higher the percentage of the whole population covered with ITNs, the greater the mosquito kill, thus benefiting both individuals using ITNs and others who sleep nearby (Hawley *et al.,* 2003).

Recent studies underscore that the poorest in Africa have so little cash that, if they pay commercial prices for ITNs, they would have no cash for essential items such as user fees for primary schools and health facilities, and even food (Guyatt *et al.,* 2002). Therefore, the major challenge is the affordability of poor people particularly in African rural settings.

1.2.9 Use of ITNs to control TBRF

There are no published data or evidence on the efficacy of ITNs on vector control of tick-borne relapsing fever. ITNs are highly likely to provide protection against tick biting and TBRF: it was observed that following the introduction of bed-nets for malaria prevention during 1995 in Mvumi area, a 67% reduction in paediatric admissions for TBRF was seen in the following year in treated areas while no effect was seen in control villages (Talbert, *personal communication*). Laboratory trials have demonstrated a high and stable acaricidal activity of lambdacyhalothrin insecticide (ICON) towards *Ornithodoros papillipes* ticks. The minimal dose of 30 mg/m^2 of ICON

on filter paper resulted in100% death of all developmental stages, remaining effective for at least 9-12 months (Vasil'eva, *et al.,* 1992).

1.3 Objectives of the Study

The study was designed and conceived with three broad objectives:

1.3.1 To explore community's knowledge, attitudes, perceptions and beliefs regarding TBRF transmission and control and how well the ITNs intervention might be received

1.3.2 To explore new insight into the natural history of TBRF vectors, *Ornithodoros moubata s.l.*

1.3.3 To evaluate efficacy of ITNs for vector control of Tick-Borne Relapsing Fever in the Randomised Controlled Trial (RCT).

It is hoped that the study will rekindle interest in a long neglected important public health problem and serious, though interesting, vector borne disease, ultimately leading to new initiatives for prevention, control or possibly elimination of TBRF from affected regions.

1.4 Ethical Approval

The ethics committees of the Tanzanian National Institute for Medical Research (Dar es Salaam, Tanzania) and Liverpool School of Tropical Medicine (Liverpool, UK) granted ethical approval of this study. Before recruiting participants, we explained the trial in details to the district and village authorities. Village meetings ('*Baraza*') were held to explain the purpose, methods and benefits of the study to the population using 'Kiswahili' (national language) and 'Kigogo' (local language). Oral informed consent was sought from the parents or caretakers of the study children. All sick study children were counseled and received free treatment at the health facility (Makulu Dispensary, 5km away from the study village).

1.5 DESCRIPTION OF THE STUDY SITE

1.5.1 The United Republic of Tanzania

The United Republic of Tanzania is located in Eastern Africa between longitude 29° and 41° East, Latitude 1° and 12° South (http://www.tanzania.go.tz/). Tanzania is the biggest of the East African countries (i.e. Kenya, Uganda and Tanzania) (See figure: 1.5.3b) with a total area of 945,000 km^2. Administratively, Tanzania has 26 regions (21 mainland and 5 Zanzibar) and 130 districts (10 Zanzibar and 120 Mainland) with a total population of 34 million people (about 51% women; 49% men and 46% are under age 15). About 50% of the population live below the poverty line with estimated Per Capita GNP at USD 246 (2001) (United Republic of Tanzania, National Census, 2002).

Tanzania has a tropical type of climate. In the highlands, temperatures range between 10°C and 20°C.during cold and hot seasons respectively. The hottest period spreads between November and February (25°C – 31°C) while the coldest period occurs between May and August (15°C – 20°C). Two rainfall regimes exist over Tanzania. One is unimodal (December - April) and the other is bimodal (October -December and March - May). The former is experienced in Southern, South-west, Central and Western parts of the country, and the later is found to the North and Northern coast. In the bimodal regime the March - May rains are referred to as the long rains or 'Masika', whereas the October - December rains are generally known as short rains or 'Vuli' (http://www.tanzania.go.tz/).

1.5.2 Dodoma Rural District

Dodoma Rural District (6°, 30' to 8°0'S, 35°, 30' to 37°0'E) in central Tanzania is located in the central plateaus at an elevation of about 800-1200m above sea level (See figure: 1.5.3c). The district is made up of 8 divisions, 48 wards, and 128 villages covering an area of 14,004 sq km. The district population is 495,176 made up of 96,686 households. The district is served by 81 health facilities (1 hospital, 6 health centres and 73 dispensaries). On average a person in the district needs to walk about 2-10 km to the nearest health facility. The nearest and furthest health facilities are 37 km and 145 km from the district capital, respectively (Mboera *et al.*, 2005).

Dodoma rural district consists of a number of mountain chains, between which are low-lying flat areas. A number of depressions are associated with these lower areas, which are generally waterlogged during the rainy season and have a tendency of salinity because of their limited outflow and are locally known as *Mbuga*. There is one river system, with Bubu River originating from the mountains in the north and flows southwards into the Bahi Swamp (http://www.tanzania.go.tz/).

The district has a dry Savannah type of climate characterized by a long dry season lasting between April and November. The average annual rainfall is 500-800mm, which is normally a short single wet season lasting between December and March. Temperature in the district varies according to altitude but generally the average maximum and minimum is 31°C and 18°C respectively. In June – August, temperatures are at times very high with hot afternoons up to 35°C and chilly nights on hilly areas down to 10°C (Mboera *et al.*, 2005).

1.5.3 Muungano Village

The study was conducted in Muungano village (6°37'0S, 37°16'0E) located approximately 12km North East of Mvumi hospital, which is 40km southeast of the Tanzanian state capital in Dodoma (figure 1.5.3b). The village has a total population of 7,500 people distributed in 15 hamlets with 1,667 households (United Republic of Tanzania, National Census, 2002). The village is bounded with three villages namely, Mvumi Makulu to the northwest, Mzula to the northeast, and Ilolo to the southeast (Figure 1.5.3d). Majority of people in the village belongs to the Gogo tribe and a small proportion of them belong to other tribes such as Masai, Beribagi and Nguu. More than 90% of the houses in the village are typical traditional made of mud flat roof with loose soil floors commonly known as *"tembe"* (See figure 1.5.3a). The common languages used by the indigenous people in this area are "Kigogo" and "Kiswahili". About 95% of the people in the village are subsistence farmers (90% peasants and 5% pastoralists). They cultivate sorghum, millet, cassava, and groundnuts, while the pastoralists keep pigs, cattle, goats, sheep, chicken and donkeys.

Figure 1.5.3(a): A typical traditional ("Tembe") house in the study site

Figure 1.5.3 (b): Map of Tanzania showing the study region

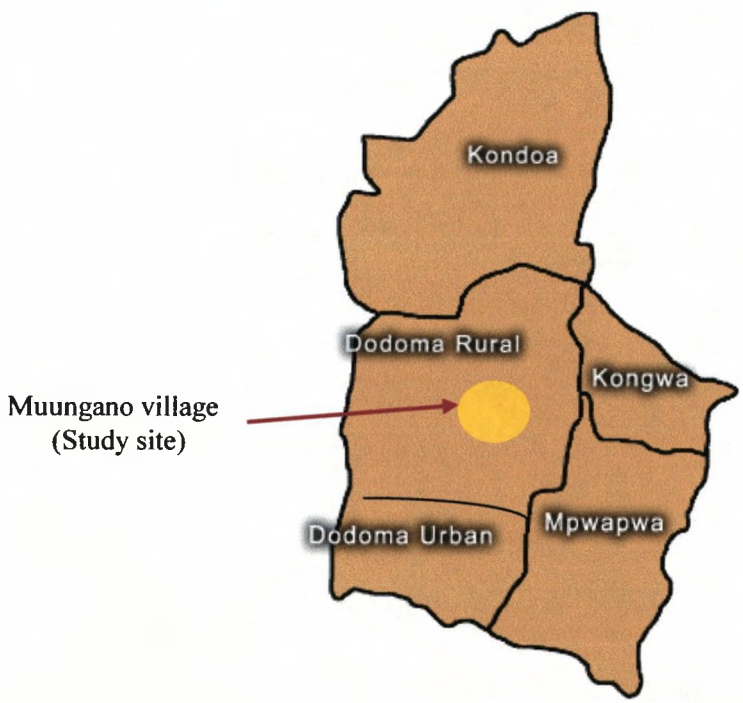


Figure 1.5.3(c): Map of Dodoma region showing the study district and the study village

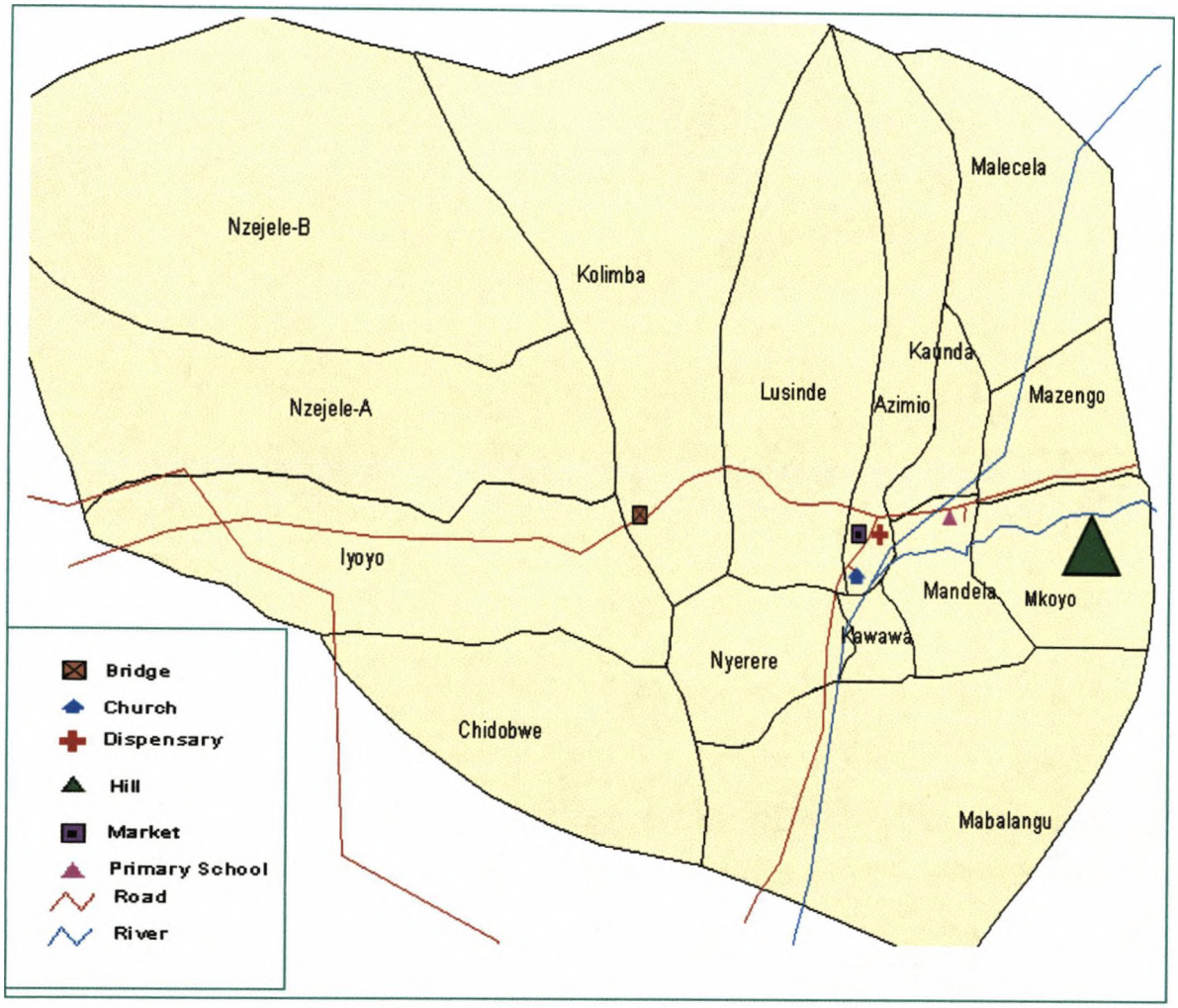


Figure 1.5.3 (d): Map of Muungano village showing the study sites

(Source: GIS map produced in 2002 by W. Kisinza)

CHAPTER 2

NATURAL HISTORY OF *ORNITHODOROS MOUBATA sensu lato*

2.1 INTRODUCTION

Information on the natural history (biology, ecology and behaviour) of the *O. moubata* complex is an essential prerequisite for designing and implementing any vector control interventions for TBRF. Little has been carried out since Walton's classic studies in the middle of the last century and much still remains unknown. To reiterate, although this vector is clearly a species complex (Walton, 1962), the identity of the forms and their geographical distribution have never been confirmed; consequently, rather than discuss or refer to different [unconfirmed] forms or species/subspecies here, all existing records and all references to the vector are referred to as *Ornithodoros moubata sensu lato.*

O. moubata s.l. are confined to African tropical regions and show a preference for natural ground in the burrows of wild porcine, and in human and domestic pig habitations, especially in the cracks, cervices and loose floors in powder-fine loamy soils in African traditional huts (Walton, 1962; Kitchen 1972). Regarding feeding behaviour, it is known that *O. moubata s.l.* feeds on the host in less than 30 minutes and that after feeding, the ticks return to the floor of the house (Geigy 1968). Nymphs feed every 3 weeks and the adults every 6 weeks (Geigy 1968, Walton, 1957 & 1962). However, there are no data on the nocturnal biting cycles, and there are still many unknowns regarding the host range of domestic populations. There is also little or no information in the literature on the distribution of *O. moubata s.l.* within tick-infested houses, on the movement of ticks between human and animal houses or on seasonal changes in tick biting patterns and intensities. Clearly, these data are important, not least because they affect the ability of ITNs to protect inhabitants of houses from bites and the likelihood that this, or any other intervention, will control TBRF.

Moreover, the number of nymphal stages in development has never been determined with certainty (Varma, 1962; Geigy, 1968; Kettle, 1984; Service, 2004). As it is not possible to distinguish the different developmental stages of *O. moubata* ticks, a simple practical classification technique based on tick length is required in order to permit a basic level of measurement of the effect of an intervention on the age structure of the domestic population of a household. Similarly, determining both the distribution of *O. moubata s.l.* within infested-households and the nocturnal cycle of tick blood-feeding activity is required. Since *O. moubata s.l.* is a species complex some of which are zoophilic or anthropophilic to different degrees (Walton, 1962), it was also considered important to determine if these local TBRF vector species would feed on hosts other than man. As more than 65% of the households in the study site keep chickens indoors at night (Gledhill, 2004), we conducted simple laboratory experiments to determine if *O. moubata s.l.* would feed on chickens or if the population of ticks inhabiting pigpens would also enter houses and feed on humans. Additionally, an insight on duration of eggs development was also investigated to determine how quickly eggs develop into the first nymphal stages as this determines the fecundity and subsequently vectorial capacity of tick vectors.

The objectives of the studies on natural history of *Ornithodoros moubata s.l.* were to determine:

2.1.1 a simple protocol for classifying tick stages

2.1.2 distribution patterns of *O. moubata* ticks within houses

2.1.3 the nocturnal cycles of blood-feeding activity of ticks on humans

2.1.4 movement of ticks between human houses and pigpens

2.1.5 propensity of ticks from human houses to feed on chickens

2.1.6 Duration of egg development into first nymphal stage

2.2 MATERIALS AND METHODS

2.2.1 Classification of developmental stages of *Ornithodoros spp.*

Ticks were collected from randomly selected houses and returned to the laboratory. By simple visual judgement, each tick was allocated to a group containing ticks of similar size and placed in a Petri dish. Each group was then re-examined to confirm that only one homogeneous group of ticks of indistinguishable size existed within. The ticks were then measured and the cut-off sizes for each stage determined. These classifications were then used in all subsequent studies.

2.2.2 Development of a protocol for examining houses for tick infestations

Prior to all of the studies on tick infestations in households, development of a reliable sampling protocol was required. A simple method involving collection and sieving of a specific quantity of soil samples was developed.

The efficiency of this protocol in recovering ticks was first validated in the laboratory. A total of 800 ticks (200 ticks, 50 of each developmental stage, in four repeated experiments) were collected from houses in Mvumi village, placed in a glass beaker (750ml) containing tick-free soil (225ml) and left to settle for l hour. The soil from the beaker was then passed through coarse (2 mm) and then through fine (0.5 mm) mesh sieves so that to be able to catch all the very small first tick stages and all ticks recovered from both sieves were collected into the collection tubes. In the laboratory, all ticks were counted and graded into their respective developmental stages and the percentage of ticks recovered calculated.

In order to maximize the recovery of ticks from the sandy soils, an additional step in the above procedure was evaluated. After passing through both sieves, the remaining sieved soil was poured into a pot (225 cm^3) and a saturated salt (NaCl) solution added

to cover the soil by 2cm. Contents were then stirred to dislodge any remaining ticks and allowed to stand for 5 minutes. The fluid was carefully poured into a large shallow tray ensuring that all of the loose materials floating on the surface were transferred and examined under a dissecting microscope for the presence of ticks. A total of 800 ticks were used in four repeated experiments (200 ticks each, 50 of each developmental stage).

2.2.3 Distribution of *Ornithodoros* ticks in the infested households

Surveys were conducted between October and November 2002 in Muungano village to determine the distribution pattern of domestic ticks within houses. A total of 200 households were randomly selected from the village. Before tick surveys (collection of ticks) were conducted, the householders from each selected households were first asked to show where they normally sleep (classified as bedrooms), where they take rest or seat before going to bed (classified as seating areas), where cooking activities take place (classified as kitchen) and where chickens are normally kept at night (classified as poultry areas). Based on this classification, four sampling sites within the houses were designated as bedrooms[2], seating areas, kitchen and poultry areas.

From each selected household, floors were sampled using a trowel, which collected a standard volume of approximately 100ml (by inserting the trowel to a depth of 2cm beneath approximately $50cm^2$ of surface). Four soil samples were taken from each sampling site within the house and transferred into a white plastic tray. In total, 16 samples were conducted from each surveyed household. The soil samples were taken outside in the daylight and examined, and all visible ticks were collected and stored in the labelled tubes. To be sure that all ticks were collected, the soil samples were sieved through course mesh (2 mm), and fine-mesh sieves (0.5 mm), and all ticks collected as described (see section 2.2.2). Thereafter, a brief search of dead ticks from the household floor was conducted and the householders inquired if they had seen or disposed of dead

[2] It was sometimes difficult to distinguish between bedrooms and seating areas as the same seating areas were used as bedrooms at night. In this case, the whole site was designated as bedroom (where much time is spent in the house at night hours).

ticks recently. The ticks were taken back in the laboratory at Mvumi hospital for examination and enumeration. Tick-density was calculated as mean number of ticks per infested household.

An additional study was carried out in May 2004 to investigate occurrence of ticks within walls, roofs as well as floors. Five households infested with *O. moubata s.l.* were selected. People from these households were pre-informed and written informed consent was obtained before conducting the activities.

In each household, sampling of ticks from house floors and walls was first conducted using the standard dry sieve method (see section 2.2.2). Soil samples from house floors were taken and all ticks collected into labelled collection tubes. House floors and walls were sampled by examining and collecting ticks at 30cm intervals in a grid pattern (see figure 2.2.2a). All ticks were collected into the labelled collection tubes.

After collecting ticks from soil substrates both in the floors and walls, the entire roof/ceiling was sprayed with pyrethrum as used in mosquito collection (WHO, 2003) (see figure 2.2.2b). Prior to spraying, white sheets were spread to cover the entire surface of the house floor. After 5 minutes, the roof was shaken to dislodge any ticks. The ticks that dropped on the sheet were collected and stored in collection tubes.

Figure 2.2.2 (a): Sampling of house walls for domestic tick infestations

Figure 2.2.2 (b): Spraying of the roofs/ceilings with pyrethrum in Mvumi, May 2004

2.2.4 Nocturnal biting cycles and seasonal changes of tick-biting intensities

To determine the tick nocturnal biting cycle and the effects of seasonal changes on tick-biting intensities, a study was conducted in Mvumi village between October 2003 and April 2004, during both wet and dry seasons. On each occasion, five heavily tick infested households were selected for the study. Alternating hourly, two researchers (W. Kisinza and A. Chuih) acted as baits by sleeping on the floor. Both investigators were provided with antibiotic prophylaxis (Doxycycline, *'Doryx Bio-Tab'* GeoTrust Pharmaceuticals, USA) and both wore tightly wrapped clothing open only at the wrists and neck. All ticks arriving at the sleeper were collected at hourly over 12 hours (19.00 to 07.00) and sorted by tick developmental stages and hour of collection. The procedure was repeated in each of the five households in October 2003 (dry season), December 2003 (beginning of wet season) and April 2004 (wet season). Biting rates were expressed as number of ticks per person per hour, based on these 15 night samples.

2.2.5 Movement of ticks between households and pigpens

Validation of marking techniques

Marking of ticks was conducted in laboratory at Mvumi hospital in September 2004. Two marking methods were first tested to determine which provide durable marks on ticks. Durability of the paint (Humbrol enamel paint; London UK) and its effect on moulting (shedding the marked skin) and survival, were determined by marking a total of 400 ticks (100 of each developmental stage marked with a 2 - 4 mm^2 red spot) and maintaining them in 1litre sand-filled glass beakers (100 ticks/ 300ml sand) in the laboratory for 14 days (see figure 2.2.5a). The persistence of fluorescent dusts (Swada Ltd: Manufacturers of fiesta fluorescent pigments, London UK) on each tick stage was also confirmed using the same procedure. The yellow fluorescent dust was dusted onto the dorsal surfaces of ticks. A control group of 400 unmarked ticks were maintained under identical conditions. After 14 days all ticks removed and examined for marks.

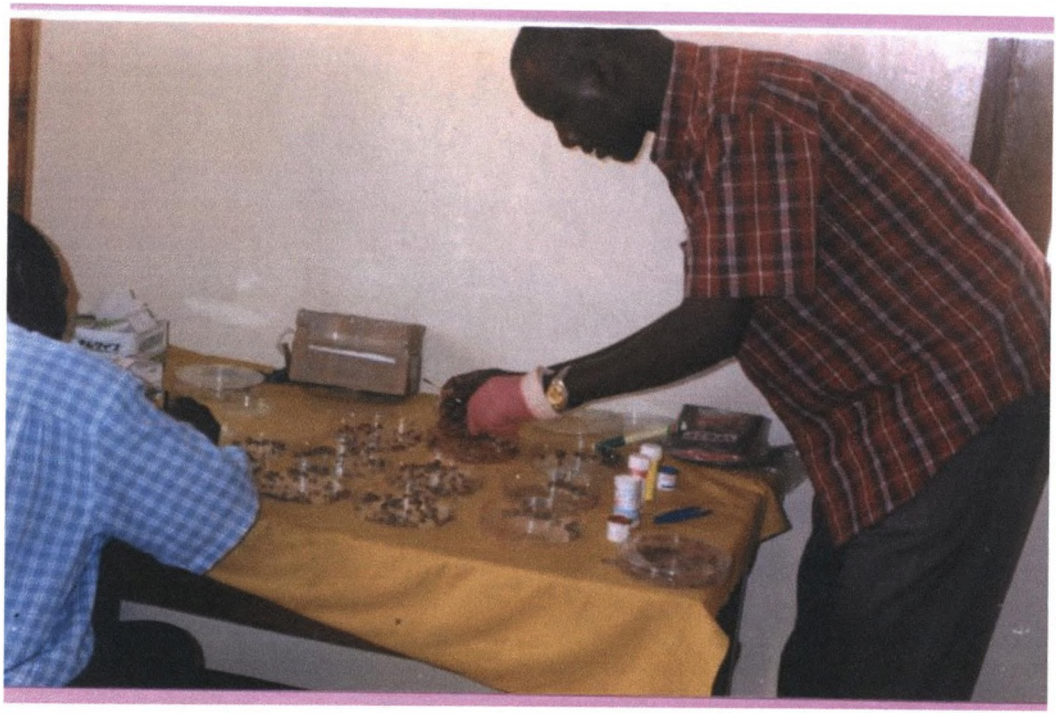


Figure 2.2.5(a): Marking of ticks with enamel paints in laboratory at Mvumi Hospital, September 2004

Mark-release experiments

In October-November 2004 in Muungano, three 'tembe'-style households with tick infestations in both human habitations and associated pigpens were selected. The three houses were in different parts of the village and at least 300 metres apart. House 1 (Figure 2.2.5b) had a pigpen (2 pigs) adjoining the house but separated from the human-inhabited (12 persons) rooms by a room holding 2 goats and 1 sheep. In houses 2 (Figure 2.2.5c) and 3 (Figure 2.2.5d), the pigpens were separate constructions at 0.75m (6 pigs) and 1m (4 pigs) from the houses (7 and 9 persons), respectively. Bedrooms were never the closest rooms to the pigpens, as day or other rooms, vacant at night, lay between. As usual, chickens roosted in these houses during the experimental period.

Ticks were collected from heavily infested households in nearby Mvumi village and marked in the laboratory. Ticks were released in pigpens only; for ethical reasons, no

marked ticks were released in the human habitations. To reduce predation by chickens and pigs, marked ticks were released after dusk (18.45hrs). A total of 1,000 marked ticks (250 of each stage) were released onto the floor in each pigpen. Sampling to recapture ticks was carried out daily at 15.00hrs, beginning the day after release and continued for 30 days, following the developed standard technique of recovering ticks from houses (see section 2.2.2). A total of 10 soil samples were randomly sampled from the pigpen and from each room of the house.

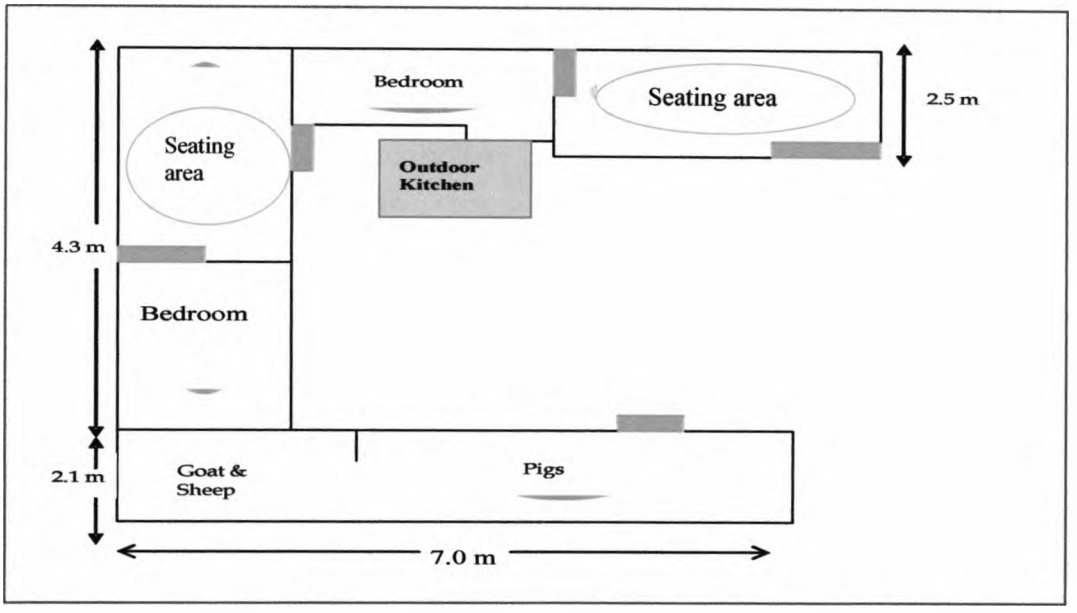


Figure 2.2.5(b) Plan of house No. 1 used in mark-release and recapture experiments

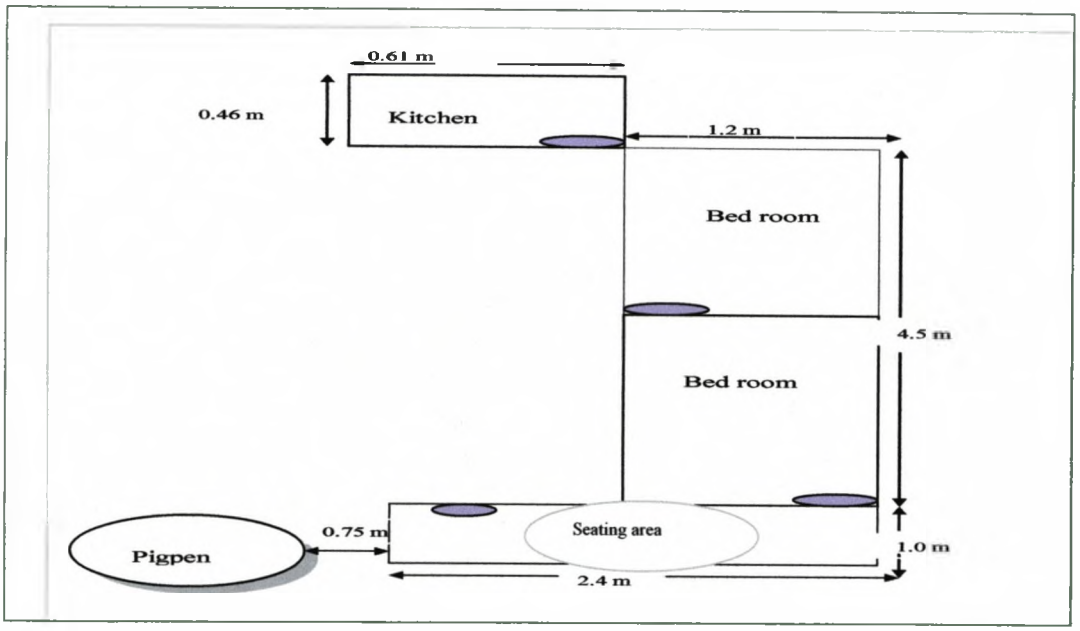


Figure 2.2.5(c) Plan of house No. 2 used in mark-release and recapture experiments

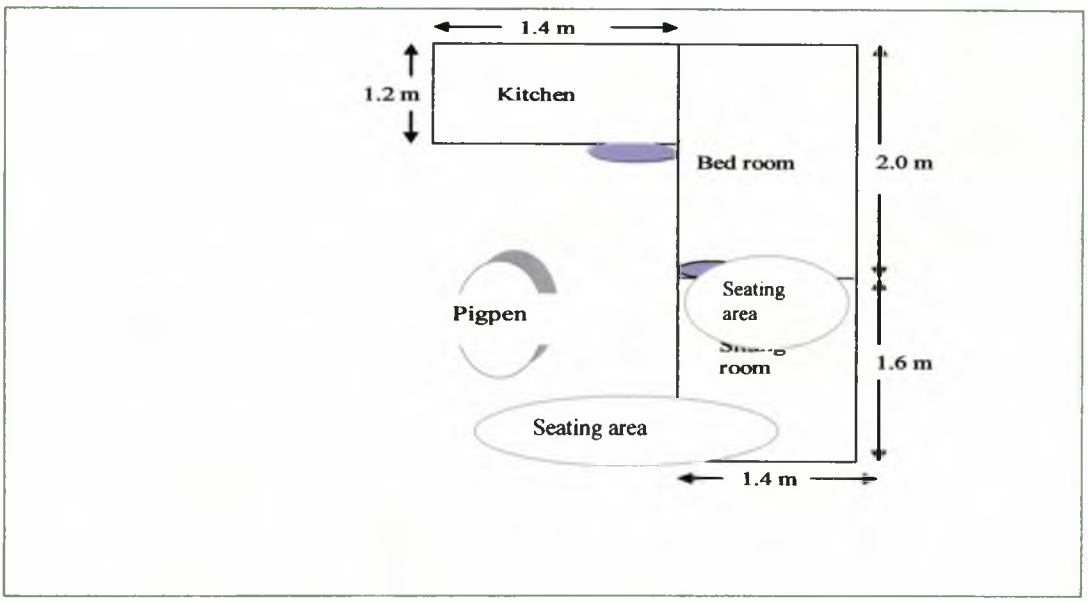


Figure 2.2.5(d) Plan of house No. 3 used in mark-release and recapture experiments

2.2.6 Feeding of *O. moubata* species on domestic chickens

Since virtually every household maintained chickens inside the home by night, chickens were a potential blood-meal source for the ticks in the house. We investigated whether ticks would accept chickens as hosts. In the laboratory at Mvumi hospital in April 2004, three cardboard boxes (40 cm x 30 cm x 30 cm) were filled to a depth of 6cm with sandy tick-free soil and 200 unfed soft ticks (50 ticks each stage), harvested from heavily infested households, were released inside each and maintained unfed in the laboratory for seven days before use, until any recently ingested blood was no longer visually detectable. At 19.00 hours on the seventh day, a chicken was introduced into each of two boxes. The third control box contained no chicken. After eleven hours, before the chickens were active, all ticks were collected from the boxes and individually squashed onto Whatman® filter paper (3M, Maidstone, UK) to examine for fresh blood. The experiment was repeated twice.

Informed consent was obtained from the owners of pigs and chickens for the mark - release experiment and for feeding experiments respectively.

2.2.7 Duration of egg development into first nymphal stage

Experiments were conducted between January and February 2005 in the laboratory at Mvumi hospital to determine duration of egg development into first nymphal stage (stage 1). Filter paper (Whatman® 3M, Maidstone, UK) was used to cover the entire base of each of six petri dishes (8cm diameter) and 10 engorged (assessed visually) soft ticks were placed in each of the three Petri-dishes. Ten unfed adults were placed in each of other three Petri-dishes. Lids were securely fitted and the dishes were placed in the dark in the laboratory. Using a hand lens (10 xs), the Whatman paper in each petri-dish were monitored for 28 days consecutively and the numbers of eggs laid and tick nymphs counted and recorded. A daily record of temperature and relative humidity was taken at 0800 hours.

2.3 RESULTS

2.3.1 Classification of developmental stages of *Ornithodoros spp.*

A total sample of 485 ticks were collected from three tick-infested houses in Muungano village in October 2002 and measured in the laboratory at Mvumi hospital to determine length (mm) distribution of ticks in the sample population. Based on tick length, ticks were classified into four developmental stages [*stage 1 (< 3mm), stage 2 (3.1 – 6mm), stage 3 (6.1-9mm)* and *stage 4/adult stage (> 9mm)* as shown in table 2.3.1 below.

Table 2.3.1: Classification of tick-developmental stages based on tick-lengths (October 2002)

Groups of ticks (tick stages)	Tick-lengths	No. of ticks (N = 485)
1	< 3mm	57
2	3.1 – 6mm	118
3	6.1 – 9 mm	252
4	> 9mm	58

2.3.2 Validation of tick-sampling technique

More than 96% of all released ticks were recovered in the validation experiments (1547/1600, 96.67%). Although the recovery rate was slightly higher by the saturated salt method (784/800, 98%) than with the simple dry sieve method (763/800, 95.38%) this was not significant (χ^2Chi-square test: $p > 0.05$). The saturated salt solution was more efficient at collecting the smaller early tick stages, increasing the recovery rates from 89.5% to 99.5% for stage 1, although the difference was not significant (Chi-square = 3.497; P>0.05). The simple dry sieve method was selected therefore, and used throughout the study as it was easier to use in the field than the more time consuming saturated salt method.

Table 2.3.2 (a): The recovery rates of ticks from soil substrates using a simple dry sieve method

Expt. No	Developmental stages (N = 50 of each stage)				Total number of ticks	
	1	2	3	4	tested	Recovered
1	47 (94%)	48 (96%)	49 (98%)	50 (100%)	200	194 (97%)
2	45 (90%)	47 (94%)	48 (96%)	49 (98%)	200	189 (94.5%)
3	45 (90%)	49 (98%)	49 (98%)	50 (100%)	200	192 (96.5%)
4	42 (84%)	46 (92%)	50 (100%)	50 (100%)	200	188 (94%)
Total	179 (89.5%)	190 (95%)	188 (94%)	199 (99.5%)	800	763 (95.38%)

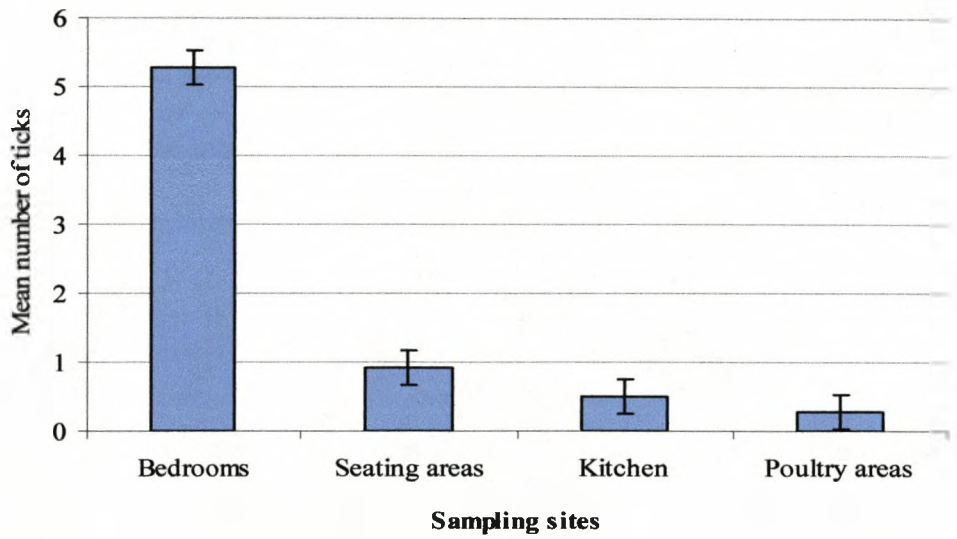
Table 2.3.2 (b): The recovery rates of ticks from soil substrates using the saturated salt method

Expt	Developmental stages (N = 50 of each stage)				Total number of ticks	
No.	1	2	3	4	tested	Recovered
1	49 (98%)	50 (100%)	49 (98%)	50 (100%)	200	194 (97%)
2	50 (100%)	49 (98%)	48 (96%)	49 (98%)	200	189 (94.5%)
3	50 (100%)	50 (100%)	49 (98%)	50 (100%)	200	192 (96.5%)
4	50 (100%)	49 (98%)	50 (100%)	50 (100%)	200	188 (94%)
Total	199 (99.5%)	198 (99%)	188 (94%)	199 (99.5%)	800	784 (98%)

2.3.3 Distribution of *O. moubata s.l.* within human habitations

Surveys on domestic tick infestations and distribution of ticks within infected households were conducted in 200 households randomly selected in the study site in October 2002. The results showed that 71.5% (143/200) of the households were infested with *Ornithodoros* ticks. Distribution of ticks within infested household was highly heterogeneous. The highest percentage of ticks was distributed in bedrooms (75.93%, 754/993) at a mean number of ticks per infested bedroom of 5.27±1.802 (SD). Other parts within houses were seating areas (13.19%, 131/993) at a mean number of ticks per infested seating area of 0.92±0.313 (SD), poultry areas (7.05%, 70/993) at a mean number of ticks per infested poultry area of 0.27±0.091 (SD) and kitchen (3.83%, 38/993) at a mean number of ticks per infested kitchen 0.49±0.167 (SD).

The difference in distribution of mean number of ticks per infested household between bedrooms and other sites within houses was significant (Fisher's exact test: $p < 0.01$). Excluding bedrooms, differences in the mean number of ticks per infested household between sites were not significant ($p > 0.05$).



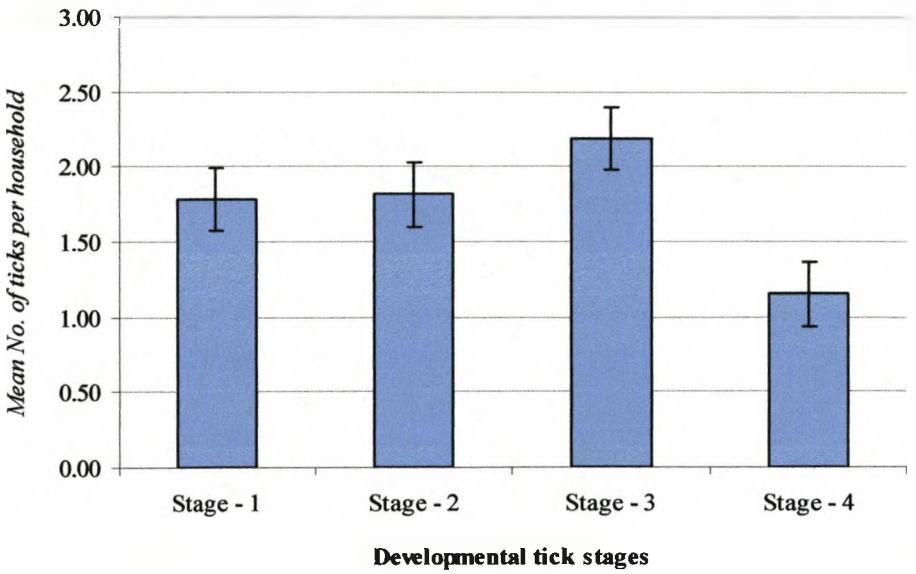
[3]Figure 2.3.3(a): Mean number of ticks per site within households (October 2002)

[3] Bars showing ± standard errors of mean (SEM) [i.e. index of reliability that expresses variation between observers and other causes of differences between repeated observations]

Table 2.3.3 (a): Mean numbers of ticks per site within households (October 2002)

Sites	Total no. of ticks in the infested households	Mean no. of ticks per infested household	SD
Bedrooms	754	5.272	1.802
Seating areas	131	0.916	0.313
Kitchen	70	0.4895	0.167
Poultry areas	38	0.2657	0.091
Total	993	6.9440	2.373

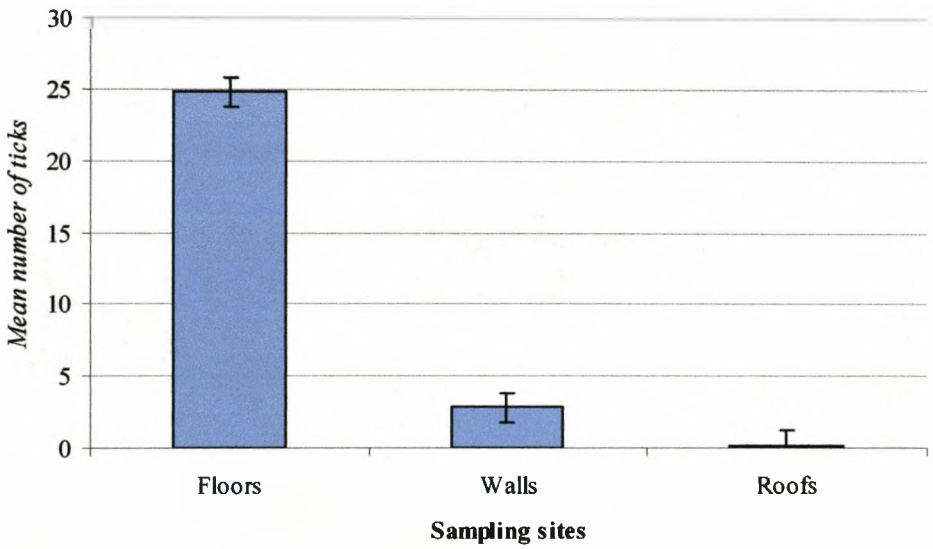
The overall mean number of ticks per infested household was 6.94± 2.373 ticks. There were more stage 3 ticks per infested household (2.19±0.143) than other tick stages, although this was not significant (Fisher's exact test: $p > 0.05$). The small sized ticks (first stages) probably hide deeper in the soil floor than other tick stages in such that some of them could not be captured during sampling of soil substrates in the floors.



[4]Figure 2.3.3 (b): Mean number of ticks of different development stages per infested household

[4] Bars showing ± standard errors of mean (SEM)

Distribution of ticks in the walls, floors and roofs from five infested households was also determined. The results showed that the highest mean number of ticks per infested household was found in floors (24.8 ± 6.380). Other sites were in walls (2.80 ± 1.218) and roofs (0.2 ± 0.224). Two ticks (stage 2) were found in infested roofs. The highest mean number of ticks sampled from 5 households was in first stage where the mean number of ticks was 10 ± 9.476 (SD) others were stage 2 (7.760 ± 5.983); stage 3 (5 ± 3.240) and stage 4 (1.60 ± 1.517). Age (size) structure of ticks in the sample population in the 3-dimentional distribution of ticks in the infested households was different from the overall age distribution of ticks in the sample population collected using the simple standard dry sieve method where the highest mean number of ticks was found in the third tick stage. The difference is presumably caused by sampling method used in the 3-dimentional distribution of ticks (pyrethrum spray catch method).



[5]Figure 2.3.3 (c): Mean number of ticks per site in infested households in Mvumi village

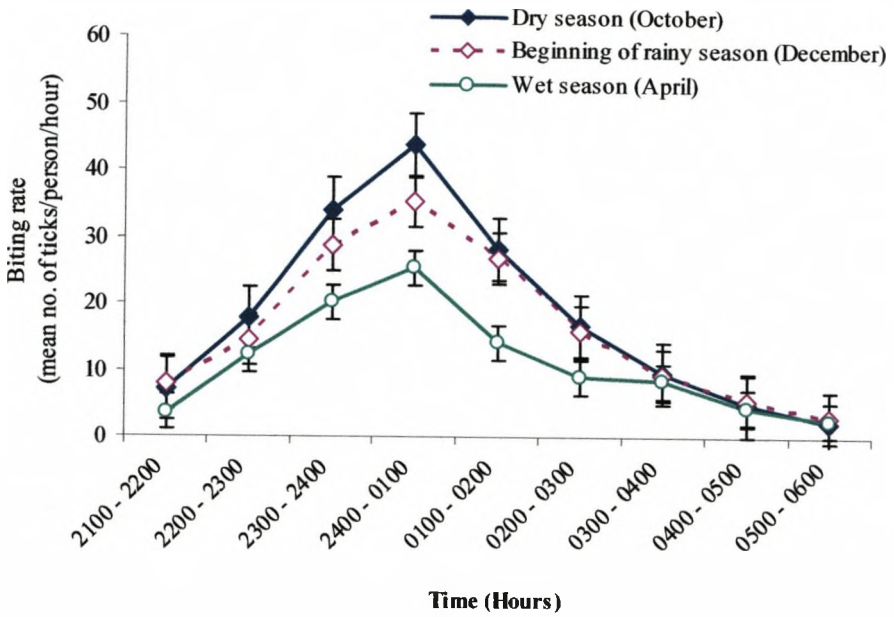
[5] Bars showing ± standard errors of mean (SEM)

Table 2.3.3 (b): Mean number of *O. moubata s.l.* per site in five infested households in Mvumi village (May 2004)

	Floors		Walls		Roofs	
	Mean No.	*SD*	*Mean No.*	*SD*	*Mean No.*	*SD*
Stage-1	10.60	9.476	0.60	1.342	0.00	0.000
Stage-2	7.60	5.983	1.00	1.222	0.20	0.447
Stage-3	5.00	3.240	0.20	0.447	0.00	0.000
Stage-4	1.60	1.517	1.00	1.732	0.00	0.000
Total	24.8	6.380	2.80	1.218	0.20	0.224

2.3.4 Nocturnal biting cycles and intensities of *O. moubata s.l.*

Nocturnal biting patterns of *O. moubata s.l.* between dry, wet and beginning of rainy seasons were similar: *O. moubata s.l.* began host seeking as early as 21:00 hours at low biting rates and increased gradually, peaking at midnight (2400 hours); thereafter the biting rates dropped gradually to zero (See figure 2.3.4). Mean numbers of ticks per person per hour at the optimal biting rates (midnight) were 43.8 ± 3.817; 35.4 ± 2.751 and 25.4 ± 1.974 for dry season, beginning of rainy season and wet season respectively (table 2.3.4). Highest nocturnal biting intensities were during dry season (October). However, biting intensity at the beginning of rainy season in December was higher than during wet season (April) (Figure 2.3.4). The difference in biting intensity between dry season (October) and wet season (April) was significant (Poisson regression test: $p < 0.001$). The difference in biting intensity between dry season and the beginning of rainy season (December) was not significant ($p > 0.05$).



[6]Figure 2.3.4: Nocturnal biting cycles of *O. moubata s.l.* (mean number of ticks per person per hour) attempting to feed on man in three different seasons in 15 different households in Mvumi village (2003 and 2004)

Table 2.3.4: Tick-biting rates (mean number of ticks per person per hour from 15 different households at different seasons in Mvumi village (2003 and 2004)

	Oct-03 *(Dry season)*		Dec-03 *(beginning of rainy season)*		Apr-04 *(Wet season)*	
Time (hours)	Mean No.	SD	Mean No.	SD	Mean No.	SD
2100 - 2200	7.2	0.627	8	0.622	3.6	0.280
2200 - 2300	17.8	1.551	14.6	1.135	12.2	0.948
2300 - 2400	34	2.963	28.8	2.238	20.2	1.570
2400 - 0100	43.8	3.817	35.4	2.751	25.4	1.974
0100 - 0200	28.2	2.458	26.8	2.083	14.2	1.103
0200 - 0300	16.6	1.447	15.8	1.228	9	0.699
0300 - 0400	9.6	0.837	9.4	0.731	8.4	0.653
0400 - 0500	4.8	0.418	5.4	0.420	4.4	0.342
0500 - 0600	2.2	0.192	3	0.233	2.6	0.202

[6] Bars showing ± standard errors of mean (SEM)

2.3.5 Movement of O. moubata s.l. between households and pigpens

Validation of marking- technique

A substantial proportion (255/400, 68.75%) of ticks marked with fluorescent dusts lost their markings during the trial (after 14 days). An additional 20 ticks (5%) either died (2.5%) or moulted (2.5%), bringing total losses of marked ticks by this method to 68.75%. Conversely, a total of only 32/400 (8%) painted ticks were lost either through mortality (8/400, 2%), moulting (9/400, 2.25%) or lost marks (15/400, 3.75%) using enamel paints. This loss through enamel paints was significantly lower than the total loss through fluorescent dusts (Fisher's exact test: $p < 0.001$). The total loss either through moults (2.25%, 9/400) or death (2%, 8/400) excluding lost marks (3.75%, 15/400) was statistically not significant different from the total loss in the control [either through moults (2%) or deaths (0.5%)] (Fisher's exact test: $p = 0.240$).

Table 2.3.5 (a): Losses through lost marks, moulting or death by enamel painting and fluorescent dusting techniques after two weeks in laboratory tests at Mvumi hospital (October 2002)

	Fluorescent dusts (N = 400)		Enamel paints (N = 400)		Control (Not marked) (N = 400)	
	No.	*%*	*No.*	*%*	*No.*	*%*
Lost marks	255	63.75	15	3.75	-	-
Moulted	10	2.50	9	2.25	8	2.00
Died	10	2.50	8	2.00	2	0.50
Total	275	68.75	32	8.00	10	2.50

Mark-Release and Recapture Experiments

Of the 2,975 marked ticks that were released into the pigpens, 775 (26.05%) were recaptured. Majority of ticks (755, 97.4%) were recaptured in the pigpens (table 2.3.5b) but a total of 20 (2.6%) were recaptured within the adjoining or nearby houses after 30 days (table 2.3.5c).

Table 2.3.5 (b): Numbers of *O. moubata s.l.* ticks recaptured in the pigpens where they were released after 30 days in Muungano village (November 2004)

	House No. 1 (N=975)		House No. 2 (N=1000)		House No. 3 (N=1000)		Total (N=2975)
	Marked	*Unmarked*	*Marked*	*Unmarked*	*Marked*	*Unmarked*	
Stage-1	133	46	75	9	153	3	419 (14.08%)
Stage-2	79	38	43	5	76	0	241 (8.10%)
Stage-3	48	23	38	2	31	1	143 (4.81%)
Stage-4	43	43	20	0	12	1	119 (4.00%)
Total	303	150	176	16	272	5	922 (30.99%)

Table 2.3.5 (c): Numbers of *O. moubata s.l.* ticks recaptured in human dwellings adjoining pigpens after 30 days in Muungano village (November 2004)

	House No. 1 (N=975)		House No. 2 (N=1000)		House No. 3 (N=1000)		Total (N=2975)
	Marked	*Unmarked*	*Marked*	*Unmarked*	*Marked*	*Unmarked*	
Stage-1	5	27	2	3	3	10	50 (1.68%)
Stage-2	5	17	1	1	1	6	31 (1.04%)
Stage-3	0	11	0	1	1	3	16 (0.54%)
Stage-4	1	17	1	0	0	1	20 (0.67%)
Total	11	72	4	5	5	20	117 (3.93%)

Of 20 (2.6%) of ticks recaptured in houses, 13 ticks (stage 1 =7, stage 2 = 2, stage 3 = 3 and stage 4 = 1) were found in bedrooms in the human habitation and had travelled at least 1.5m (measured as a direct line from the release point in the pigpen to the room of recapture). The other 7 ticks (stage 1 = 4, and stage 2 = 3) were found in seating areas after 30 days (table 2.3.5d).

Table 2.3.5 (d) Distribution of *Ornithodoros* ticks recaptured within houses after 30 days (November 2004)

Tick stages	House No. 1 (N=975)		House No. 2 (N=1000)		House No. 3 (N=1000)		Total (N=2975)
	Bedrooms	*Seating areas*	*Bedrooms*	*Seating areas*	*Bedrooms*	*Seating areas*	
Stage-1	5	1	0	2	2	1	11 (0.37%)
Stage-2	1	2	0	1	1	0	5 (0.17%)
Stage-3	2	0	0	0	1	0	3 (0.10%)
Stage-4	0	0	1	0	0	0	1 (0.03%)
Total	8	3	1	3	4	1	20 (0.67%)

2.3.6 Feeding of *O. moubata s.l.* on domestic chickens

Fresh blood was detected in 21.25% (85/400) of ticks introduced into and recovered from boxes with chickens, while no ticks from control boxes (n=200) had detectable blood. Between 16% and 25% (first and fourth stages respectively) of ticks of different developmental stages successfully fed (See table 2.3.6). Differences in feeding rates between tick stages were not statistically significant (Chi-square tests: $p > 0.05$).

Table 2.3.6: Number of ticks with detectable blood obtained from domestic chickens in the laboratory experiment at Mvumi hospital (April 2004)

Developmental stages	No. of ticks tested	No. of ticks with detectable blood
Stage-1	100	16
Stage-2	100	23
Stage-3	100	21
Stage-4	100	25
Total	400	85

2.3.7 Duration of egg development into first nymphal stage

The visually engorged (bloodfed) ticks started laying eggs in laboratory on seventh and eighth days (42 eggs) post experiment and continued to the 9th day (11 eggs). The visually unfed ticks were obviously not completely unfed as they too laid eggs. These ticks started laying eggs on the 9th (20 eggs) up to 12th day (6 eggs) post experiment (table 2.3.7a). Clearly what we considered unfed must have had sufficient blood from an earlier meal to still enable egg production.

Development of egg into nymphal stages started on the 13th day (40 nymphs) post oviposition in the fed ticks, while development of egg into nymphal stages in 'unfed' ticks started on 18th day (23 nymphs) post oviposition. Thus, duration of egg development into first nymphal stage ranged from 13 to 18 days post oviposition (table 2.3.7b).

Table 2.3.7 (a): Duration of oviposition at the optimal laboratory conditions between 'fed' and 'unfed' ticks (February 2005)

Days	No. of eggs laid per day in the 'bloodfed' ticks			No. of eggs laid per day in the 'unfed' ticks		
	Plate -1	*Plate - 2*	*Plate - 3*	*Plate -1*	*Plate - 2*	*Plate - 3*
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	42	0	0	0	0	0
8	0	0	0	0	0	0
9	27	0	0	20	0	0
10	0	0	0	0	0	0
11	0	13	0	0	0	0
12	0	0	0	6	0	0
Total	69	13	0	26	0	0

Table 2.3.8(b): Duration of egg development into first nymphal stage at the optimal laboratory conditions (February 2005)

Days	No. of nymphs per day in the 'bloodfed' ticks			No. of nymphs per day in the 'unfed' ticks		
	Plate -1	Plate - 2	Plate - 3	Plate -1	Plate - 2	Plate - 3
13	40	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0
16	20	0	0	0	0	0
17	0	0	0	0	0	0
18	0	11	0	23	0	0
Total	60	11	0	23	0	0

2.4 DISCUSSION

This is the first study to be conducted in the natural history of *O. moubata s.l.* since Walton's classic studies in the middle of the last century. Until now, there were no data on the nocturnal biting cycles and there is little or no information in the literature on the distribution of *O. moubata s.l.* within tick-infested houses, movement of ticks between human and animal houses or on seasonal changes in tick biting patterns and intensities.

It is also important to know the developmental stages of ticks as for any vector control strategy for TBRF, information on which tick stage is active and readily feed on hosts or susceptible to pyrethroid insecticides are paramount essential. As it is not possible to distinguish different developmental stages of *O. moubata* ticks, we developed a simple practical classification technique based on tick lengths.

This study has shown that it is possible to distinguish four developmental stages of *O. moubata s.l.* Varma (1962) and Geigy (1968) described the life cycle of soft ticks and reported that life stages of *O. moubata s.l* are not readily distinguishable. The six legged larva moults to the first nymphal stage before hatching and going through multiple nymphal stages, gradually increasing in size until the final moult to the adult stage. Although the present study has not attempted to differentiate between these stages, the standard protocol of classifying *O. moubata s.l.* into four 'growth' stages will be a useful tool in the absence of a method to do so.

The traditional method of tick sampling from the infested households has been described before (Walton, 1962) where the soil samples were taken from the house floors and examined for tick infestations. We developed a standard quantitative method of recovering domestic ticks from tick infested households. The results showed that both (simple dry sieve and saturated salt) methods are effective in recovering soft ticks from soil substrates, although the saturated salt method is more efficient at collecting the smaller early stages of ticks than the simple dry sieve method. The latter is much

easier to use in the field than the time consuming saturated salt method, particularly in a large scale survey. The dry sieve method was therefore adopted throughout this study.

There is some information in the literature on the distribution of *O. moubata s.l.* within tick-infested houses. Walton (1957) described the spatial distributions of *O. moubata s.l.* ticks in human dwelling in Tanzania and maintained that distribution of ticks was limited by the extremes of microclimatic variation found in the houses including temperature and relative humidity. Ticks were not found in the wet and cool grounds of the houses. Domestic tick infestation occurs at temperatures that range from 20°C to 31°C and the Relative Humidity from about 60% to 90%. It has been also reported that certain biochemical such as carbon dioxide as well as heat and movement serve as stimuli for host seeking behaviour of soft ticks (Sonenshine, 1993), and that distribution of ticks was associated with the presence of domestic animals in houses (Walton, 1967), but there is no information in the literature on distribution of ticks within infested houses.

Our findings clearly showed that distribution of *O. moubata s.l.* ticks within the infested houses is heterogeneous. The high numbers of *O. moubata s.l.* are distributed in bedrooms, presumably as ticks remain in close proximity to the main source of blood meals. The distribution of ticks in the bedrooms gives an advantage to using ITNs as they are used to protect people from tick-bites. Several authors suggested that soft ticks are attracted by the emanations and warmth of the human bodies and odours (Burgdorfer, 1969&1999; Parola, 2001), but this, although very likely, remains unproven. Most blood-feeding arthropods are attracted to their hosts by a combination of cues, including CO_2, odour and temperature (Pates & Curtis, 2005), Thus, as for mosquitoes, people sleeping under treated nets might act as bait for ticks, facilitating their contact with the insecticides treated on netting.

This study also showed that domestic tick infestation in Muungano village was very high, with infestations ranging from 50% to 87.5% with a higher number of ticks in stage 3 than other tick developmental stages. The reason for the higher number of ticks

in stage 3 than adult stages is probably that large adult ticks are easily visible and much vulnerable to predation. It is also likely that the small stages were not captured probably they were in deeper floor.

Heavy domestic tick infestations in some parts of Africa have often been reported. Domestic tick infestation rates of 80% were reported in 1953 in Kahama district South of Lake Victoria in Tanzania (Walton, 1967). Trape (1991) reported domestic tick infestation rates of 66% in Senegal. High infestation rate was also reported recently in Dodoma central Tanzania to be as high as 88% (Talbert *et al.,* 1998). Here we found high domestic tick infestations and high nocturnal biting intensity peaking at midnight. Also high distribution of ticks was found in the bedrooms in the infested households. This suggests that people in the region are at high risk of transmission with *Borrelia* spp. infections as high proportion (42.5%) of *Ornithodoros* ticks in the region are infected with *Borrelia* (Mitani *et al.,* 2004) and also ticks have been reported to be *Borrelia* reservoirs (McCall, 2001).

The current study has also demonstrated that *O. moubata s.l.* feeds on domestic chickens. Our results, where more than 21% of ticks introduced into the boxes with chickens were detected with fresh blood meals, clearly showed that *O. moubata s.l.* feeds on domestic chickens. All tick stages readily and equally fed (all tick stages can transmit *Borrelia* pathogens). Walton (1964) reported in Kenya that ticks could readily feed on domestic chickens to the almost total exclusion of man.

The recent study conducted in Mvumi by Motshegwa (2004) showed that 11.7% of domestic chicken in the area were infected with *Borrelia* spp. Given that chickens can also harbour *B. duttonii* infections (Motshegwa, 2004); this is an important observation as it has shown that *B. duttonii* could be transmitted between hosts by the same vector. It is likely therefore that *O. moubata* ticks are also found around chickens because they feed on chickens. Phipps (1950) also reported that ticks were found congregated in chicken habitats in Tanzania, and suggested that it was possibly because of the warmth.

O. moubata s.l., like many in this genus, is a nocturnal feeder, but its cycle of activity has never been recorded. Our results showed that *O. moubata s.l.* host seeking begins at 21:00 hours and gradually increases, peaking at midnight (2400 hours) after which the biting rate drops gradually to almost zero at 0600 hours. The results also showed that there is seasonal variation of biting intensity, but the nocturnal biting pattern remained the same. The same nocturnal biting pattern has been reported in malaria vectors, where biting rates peak at midnight (Maxwell *et al.,* 1998; Pates & Curtis, 2005). Thus, if used by sleepers for prevention of malaria, ITNs will also be useful in protecting people from tick-bites and consequently TBRF.

Our results showed that nocturnal tick-biting occurs both during dry and wet seasons with higher biting rates during dry season than in wet season, this probably because in wet season the microclimatic condition of tembe houses becomes cool, wet and the house floor becomes compacted giving unfavourable conditions for domestic tick proliferations resulting into low tick-biting intensity. Similarly, in Meru, Kenya, Walton (1957) reported that ticks were not found in the wet and cool climate because the grounds of traditional houses (huts) were also wet. This suggests that transmission of *Borrelia* infections can occur both during dry and wet seasons with high transmission likely primarily during the dry seasons when there is high tick-biting intensities.

As more than 85% of the households in Muungano village were keeping pigs, it was important to establish if tick populations in pigpens adjoining to the human habitations constituted a separate population to those within the human house, or alternatively, if they were similar and whether ticks could re-populate the human dwelling after eradication from inside the household.

This is the first study to use mark-release experiments to determine the movement of ticks between households and pigpens. This study showed that ticks were recaptured from both pigpens and human dwellings and that ticks moved from pigpens to human dwellings across a distance of at least 1.5m. Therefore, it appears that ticks are capable of repopulating human dwellings from pigpens. The highest proportion of ticks

recaptured in the households was of the smallest size (1st stage). This suggests that small size ticks (stage-1) either were more active in locating human hosts, less vulnerable to environmental stresses or possibly were less conspicuous to predators.

This study suggests therefore that households adjoining pigpens could be at risk of tick infestations. Indeed, Motshegwa (2004) reported that 16% of pigpens in the villages around Mvumi hospital were tick infested and were more likely to be so if located close to an infested household. This shows that controlling TBRF by treating households alone may not be enough and pigpens may require treatment too to achieve sustained control.

Our results also showed that duration of egg development into first nymphal stage ranged between 8 and 11 days post oviposition. Kettle (1984) and Service (2004) reported that eggs hatched 8 days post oviposition at 30°C. Thus under normal ambient conditions eggs developed rapidly. Whether this development period is extended during period of environmental stress (such as extreme high or low temperatures and/or humidity) remains unknown.

CHAPTER 3

KNOWLEDGE, ATTITUDES, AND PRACTICES RELATED TO TBRF IN DODOMA RURAL DISTRICT CENTRAL TANZANIA

3.1 INTRODUCTION

Tick-Borne Relapsing Fever is an endemic disease in Tanzania and although national statistics do not exist yet, it is clearly the third most important vector-borne disease, possibly as prevalence as malaria and lymphatic filariasis. TBRF is reported to occur in 20% of the districts of Tanzania mainland, being endemic in central Tanzania and common in north-western and south-western parts of the country (Magesa *et al.,* 2001). However, there has never been a systematic survey conducted to establish the burden of disease in the country, nor is there any strategy for TBRF control and prevention.

As appropriate level of knowledge of vector biology, ecology and behaviour by affected communities is an important component of vector control, information on TBRF in Tanzania has never been available to the people and there are no published data in the country focussing on existing knowledge, attitudes, and practices regarding TBRF.

Indeed, community participation is often considered the most important prerequisite for the success of prevention and control programmes of any disease (Alilio *et al.,* 1998; Kengeya-Kayondo *et al.,* 1994; Winch *et al.,* 1992). Cooperation of the affected population is essential in the implementation and use of programme activities. Programme implementers need to understand the disease-related knowledge, attitudes, and practices (KAP) of the community, because these are important determinants of community participation (Singh *et al.,* 2006).

As part of an effort to involve community members in TBRF control, we therefore conducted a Knowledge, Attitudes, and Practices (KAP) study before carrying out ITN trial. The main objective of the study was to explore the local population's knowledge, attitudes, practices and beliefs regarding TBRF transmission and control and how well

the intervention might be received. Specifically, the study wanted to determine (1) household structure and sleeping arrangements, (2) level of knowledge of the cause and transmission of TBRF, (3) perceptions of TBRF symptoms, and (4) the community's practices related to TBRF prevention and health seeking behaviour.

3.2 MATERIALS AND METHODS

3.2.1 Study site

The study was conducted between October and November 2002 in Muungano village, Dodoma rural District central Tanzania. The total population of the village was 7,500 people distributed in 15 hamlets with 1,667 households with a total of 495,176 people in the district (United Republic of Tanzania, National Census, 2002). The district is served by 81 health facilities (1 hospital, 6 health centres and 73 dispensaries). A full description is presented in section 1.5.3.

3.2.2 Study population and sample size determination

The study involved 200 face-to-face interviews with heads of households[7] (males and females) in Muungano village aged 18 years and above. The heads of households were chosen as the study subjects because they play major roles in decision – making process at the household level in Tanzania.

As community's knowledge related to TBRF was not known, the maximum sample size was calculated based on the hypothesis that at least 50% of heads of households had knowledge on TBRF. The sample of heads of households was calculated according to: $n= 1.96^2 P (1-P)/e$ formula (Moore *et al.*, 1999), where n is the sample, P is the expected proportion of heads of households who had TBRF knowledge and e is the error of the estimate (± 10%). The sample n was multiplied by two because of the design effect associated with cluster sampling and this led to a sample size of 192 heads of households to be interviewed. Based on Njunwa *et al.* (1991) report of 4% dropouts of people involved in KAP studies on ITN trials for malaria control in Tanzania, we

[7] Households were defined as residential units with one or more individuals in occupation. Multiple families residing in the same household were considered one household. Multiple structures within a compound occupied by the dependents of household head were also considered one household.

therefore added 4% of the sample size (8 respondents) to supplement the expected dropouts and making a total sample size of 200.

All households were listed (enumerated) and assigned numbers. Using a simple random method, a total of 200 heads of households were selected and involved in the study. Data were collected using a pre-tested semi-structured questionnaire (Figure 3.2.2). Informed consent was first obtained from the interviewees during household surveys before conducting a face-to- face interview. Demographic characteristics of respondents were recorded and people were interviewed on sleeping arrangements (on beds or on floors, usage of nets, inside or outside house), knowledge related to TBRF transmission, symptoms and prevention. People were also asked on domestic tick problems (infestations and tick-bites), strategies used to control domestic tick infestations and health seeking behaviour. Other information such as basic animal husbandry practices (indoors or outdoors), cooking practices (indoors/outdoors) and household characteristics (loose or hard floors, plastering of the houses) were also explored.

3.2.3 Data analysis

Data were entered in the computer using Epidemiological Information (Epi-Info) data processing package and analyzed using Statistical Package for Social Sciences (SPSS for windows, version 10.0, Chicago, USA).

3.2.4 Quality Control

The questionnaire used in the study was pre-tested in Mvumi village, and then was refined to correct areas of inconsistency and ambiguity. The interviews were conducted in Kiswahili (the National language). Only one researcher (William Kisinza) interviewed all the study subjects. Before data entry and analysis, the questionnaires were checked for coding errors, completeness and consistency.

Figure 3.2.2: Questionnaire for KAP study

KNOWLEDGE, ATTITUDES AND PRACTICES RELATED TO TBRF

Section A: Socio-demographic characteristics of respondents

i. Village name: _____
ii. Hamlet name _____
iii. Hamlet leader _____
iv. House ID No. _____
v. Name of respondent _____
vi. Age _____(yrs); Sex: M/F _____
vii. Total number of people currently living in the house_____
Males [ ] Females [ ] Pregnant women [ ] Children<5 Years [ ] <1 year old [ ]
viii. Use of BED NETS in the household:
(1) Yes [ ] (2) No [ ]
If **YES**, type of bed nets used (a) treated net [ ] (b) Untreated net [ ]
ix. Use of RAISED BEDS (1) Yes [ ] (2) No [ ]

Section B: Knowledge of TBRF transmission

1. Mention top 5 diseases in the village:
(a) Malaria [ ] (b) Fever [ ] (c) Diarrhoea [ ] (d) Relapsing fever [ ]
(e) HIV/AIDS [ ] others (list them) [ ]
2. (If no RF mentioned ask), have you heard of relapsing fever? (probe for local name)
(1) Yes [ ] (2) No [ ]
3. If YES, (in Q2); How do people get relapsing fever?
(1) Tick bites [ ], (2) mosquito-bites, (3) bites by other insects (mention them)
4. Is any body in this household having a tick fever now?
(1) Yes [ ] (2) No [ ] (3) don't know [ ]
5. Has anybody in this household had tick-bone relapsing fever in the past seven days?
(1) Yes [ ] (2) No [ ] (3) Don't know [ ]
6. What are TBRF-related symptoms for people suffering from tick-fevers? _____
7. What treatments or actions were taken from people suffering from TBRF?
(1) Go to the dispensary/health facility for diagnosis [ ]
(2) Go to a traditional healer [ ]
(3) Buy medicines from medical stores and treat at home [ ]
(4) Treat at home with traditional/local medicines [ ]
(5) Do nothing [ ] (6) don't know [ ] (7) others (mention them _____
8. What traditional/local medicines do people use to treat TBRF-related infections? (Mention)
9. What do you and your family do to prevent from getting relapsing fever?

Section C: Knowledge of TBRF – vectors & domestic infestations

10. Do you have ticks inside your household? (1) Yes [ ] (2) No [ ] (3) Don't know [ ]
11. Do you or any of your family get bitten by the ticks at night? (1) Yes [ ] (2) No [ ]
12. How often do you get bitten? _____ (days/month)
13. What times of the year do you get ticks and/or tick-bites?
(1) Dry season [ ] (2) wet season [ ] (3) All year round [ ] (4) Others (mention)
14. What do you and your family do to prevent from domestic tick infestations and/ or tick-bites?

Section D: Animal husbandry practices

15. Do you keep animals at this household? (1) Yes [ ] (3) No [ ]
16. If YES (Q14): what type of animals do you keep (mention them)____
17. Where do you keep animals? (1) Indoors [ ] (2) Outdoors [ ] (3) Others [ ] Mention

Section E: Observational data

18. Type of households (traditional, Modern)
19. Conditions of house floors (hard or loose floors)
20. Cooking habits (indoors/outdoors)
21. Keeping of chickens (indoors/outdoors)
22. Plastering (rendering the households with mud etc)

3.3 RESULTS

3.3.1 Socio-demographic characteristics of the study population

The study involved 200 heads of households, and men constituted 47% of the study population, giving a sex ratio of 106 women per 94 men. Ages of respondents ranged from 18 to 70 years with a mean age of 45 years (± 0.8 SD). Of the 200 study population, 198 (99%) participated in interviews, and two dropped out. More than half of the study population was illiterate (64.3%), while 35.7% were educated to primary school level. The main occupation in this area was subsistence farming (94.9%). Other occupations included carpentry (1.5%), vendors/retailers (3.6%). Agricultural farming included cassava, finger millet and groundnut cultivation. Most of the households kept chickens (62.6%). Others kept pigs (35.4%) and cattle /goats (2%).

3.3.2 Characteristics of the households

Only 22 (11%) households had raised beds and only 8 (4%) households had bed nets. A total of 194 (98%) households had traditional style mud houses with flat roofs known as '*tembe*'. These houses were of two categories: (1) 89 (45%) of the houses were those with floor and walls rendered with mud ('hard or plastered floor'), and (2) 109 (55%) were those with loose earth floor (i.e. not rendered with mud, 'loose floor'). The houses with hard floors were further classified into two groups: (a) 37 (41.57%) were those houses with newly plastered floor (received plaster within the past two weeks), and (b) 52 (58.43%) were those with old plaster (received plasters more than two weeks before).

Table 3.3.2: Characteristics of the study households

Characteristics	No. of respondents (N=198)	Percentage
Owning raised beds	22	11.00
Owning of bed nets	8	2.00
Traditional style mud houses ('tembe')	194	98.00
Households with 'hard' floor (rendered with mud)	89	45.00
Households with 'loose' floor (not rendered with mud)	109	55.00
Households with new plastered house floors (plastered within previous two weeks)	37	41.57
Households with old plastered house floors (plaster more than two weeks old)	52	58.43

3.3.3 Beliefs regarding causes of illness

To elicit community's beliefs regarding causes of illness in the study site, people were asked to mention the top five major causes of illness in the village. Out of 198, 170 (85.87%) cited that malaria was the highest public health problem in the study site followed by tick-borne relapsing fever 120(60.6%). Other illnesses mentioned included fevers of unknown original 40 (20.1%), diarrhoea 14(7.1%), HIV/AIDS 1(0.5%) and other illnesses (coughing, eye infection, abdominal pain & pneumonia) see figure 3.3.3.

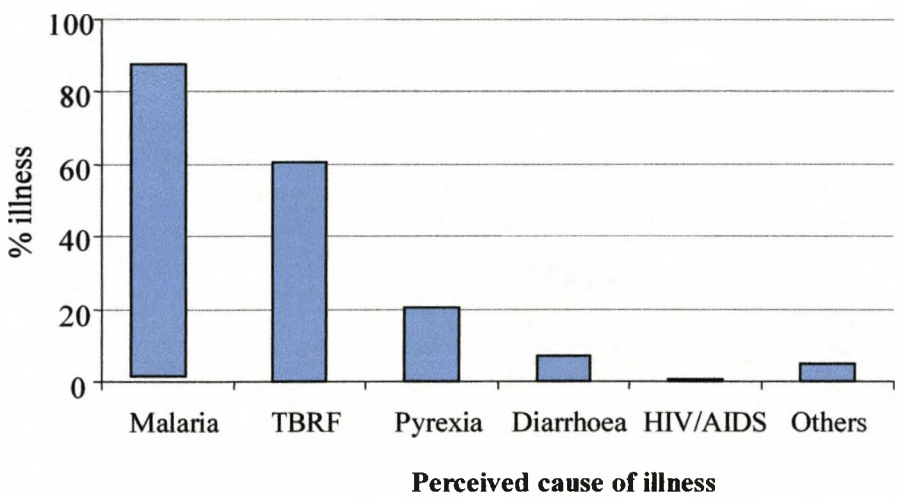


Figure 3.3.3: Main causes of illness perceived by householders in the study site

3.3.4 Knowledge of TBRF

When asked on the cause of relapsing fever, majority (85.86%) of respondents reported that it is transmitted by tick-bites. Only 7.1% did not recognise of the disease. Others reported that TBRF is caused by mosquito bites (5%) and bedbugs/fleas (2%) (See table 3.3.4).

Table 3.3.4: Knowledge on the cause of TBRF

	No. of respondents (N=198)	Percentage
Tick bites	170	85.86
Mosquito bites	10	5.05
Other insect bites *(Bedbugs & Fleas)*	4	2.02
Don't know	14	7.10

3.3.5 Knowledge of domestic tick infestations

The study showed that 82.83% of respondents reported to have had domestic tick infestations and tick-biting in their households. The majority reported that high domestic tick infestations were found during dry season. And minority (2.53%) reported to have had domestic tick infestations all year round (see table 3.3.5).

Table 3.3.5: Knowledge on domestic tick infestations in the study site

	No. Respondents (N=198)	Percentage
Reported on domestic infestations and tick-bites	164	82.83
Domestic tick infestations during dry season	169	85.35
Domestic tick infestations during wet seasons	24	12.12
Domestic tick infestations all year round	5	2.53

3.3.6 Knowledge of TBRF-related symptoms

All respondents recognised the typical symptoms of TBRF. The majority (69.5%) mentioned fever as symptom for TBRF infection. Other symptoms mentioned included body pains (8.5%), headaches (8.5%), chills or cold (4. 5%) and vomiting (3.5%).

Table 3.3.6: Knowledge of TBRF – related symptoms

	No. Respondents (N=198)	Percentage
High fever	139	69.50
Body/muscles pains	17	8.50
Headaches	17	8.50
Chills	9	4.50
Diarrhoea	9	4.50
Vomiting	7	3.54

3.3.7 Community health seeking behaviour for TBRF

The study showed that majority (84.3%) of people in the study site sought medical treatment from the local health facilities. Only 2% sought medical services from traditional healers, 12.1% of respondents reported to have used self-medication at home either using traditional medicines (9.1%) or medicines from medical stores/shops. 1.52% of the interviewees reported to have had done nothing when they got ill.

Table 3.3.7: Community health seeking behaviour for TBRF

	No. Respondents (N=198)	Percentage
Go to the health facility (dispensary)	167	84.3
Go to the traditional healers	4	2.0
Home treatment with traditional medicines	18	9.1
Home treatment with dispensary medicines	6	3.0
Do nothing	3	1.5

3.3.8 Traditional medicines used to treat TBRF

When asked on the use of traditional medicines/herbal plants for the treatment of TBRF, 60.6% (120/198) reported to have used or seen other people using medicinal plants. The most commonly mentioned traditional medicinal plants (the names are in 'Kigogo' the traditional language of the indigenous people in the area and botanical names provided into the brackets) were 'Mkakati' *(Cassia orbbreviata),* 'Mdejedeje' *(Acacia seyal),* 'Msungusungu' *(Grewia burtii),* 'Mung'wenyengule' *(Calyptrothea taiensis),* 'Mitundulu' (*Dichrostachys cinerea*), 'Mkakatika'*(Cassia orbbreviata),* "Mapanyanya' 'Mwagulwa'*(Commiphora stuhlmanni)* and Nzenye (*Sapium bussei*). All the mentioned plants were used to treat TBRF.

Table 3.3.8: Types of traditional medicines/herbal plants used to treat TBRF

Local names ('Kigogo')	Botanical names	No. Respondents (N=120)	Percentage
Mkakatika	*Cassia orbbreviata*	25	20.83
Mdejedeje	*Acacia seyal*	18	15.00
Mapanyanya	-	16	13.33
Mguluchila	*Markhamia obtusifolia*	13	10.83
Msungusungu	*Grewia burtii*	13	10.83
Mung'wenyengule	*Calyptrothea taiensis*	12	10.00
Mitundulu	*Dichrostachys cinerea*	10	8.33
Mwagulwa	*Commiphora stuhlmanni*	8	6.67
Nzenye	*Sapium bussei*	5	4.17

Source of botanical names: Monela *et al.,* (2004) (IUCN-The World Conservation Union Report, East African Region Office)

3.3.9 Control of domestic tick infestations

The majority (96.46%) of respondents reported that they plastered their house floors and walls regularly. Others (17.2%) poured or sprinkled hot water on the walls and house floors to kill ticks. Most (80%) said they regularly sweep their houses that it didn't stop domestic infestations. Other (20.7%) used insecticides other than ITNs to control domestic tick infestations in their households. The insecticides (permethrin) were bought from shops or medical stores in the village, and then the insecticides were mixed with quantity of water and mud, and then plastered on the house floors and walls; while others after mixing with water, they just sprinkled on floors and walls to kill ticks. The village no history of net use as only 8 (4%) of people in the study site owned untreated bednets (see table 3.3.9).

Table 3.3.9 Methods used by the community to control domestic tick infestations

	No. Responses (N=198)	Percentage
Plastering the house floor & walls	191	96.5
Sprinkling hot water	34	17.2
Sweeping the house regularly	158	79.8
Used insecticides other than ITNs	41	20.7
Use of bednets (untreated bednets)	8	4.0

3.4 DISCUSSION

In this study, heads of households were chosen as the study subjects because they have the decision making capacity for the households in Tanzania (Njunwa *et al.,* 1991; Mubyazi *et al.,* 2005).

The study showed clearly that TBRF is considered to be public health problem in the area as the disease was ranked as one of the top five health illnesses in the village second only to malaria. Domestic tick infestation was cited by majority (83%) of respondents to be high. This probably has been attributed by many factors such as poverty implications as majority of people in the area were subsistence farmers (90%) can not afford to construct modern houses. The results showed that 98% of the households in the areas were typical traditional style ('tembe' house) which probably encourages proliferation of domestic tick infestations. Similar findings have been also reported in Meru Kenya that traditional African households were highly tick infested compared to the modern houses (Walton, 1962).

The study also showed that majority of people (89%) in the area slept on floors using either mating blankets or animal hides. This also makes easier for domestic ticks to bite and transmit TBRF. Walton (1967) reported high domestic tick infestations in the 'tembe' households in central Tanzania and that the infestations were eradicated by improving design of houses, plastering of walls and floors, the adoption of bedsteads, removal of domestic animals and fowls from house and good housekeeping.

Knowledge about TBRF was well articulated by majority of respondents as more than 85% cited that TBRF is transmitted through tick-bites, and all study subjects reported correctly the TBRF related clinical symptoms, although could not distinguish clinically from malaria. Knowledge of TBRF might have been imparted to people seeking medical treatment at health facilities or during antenatal clinic services.

TBRF has been endemic disease in the region (Barclay and Cutler, 1990) with domestic tick infestations as high as 88% (Talbert *et al.,* 1998). In this case people might have been in contact with soft ticks and the disease for long time in such that they are knowledgeable about tick-borne relapsing fever, which confirm that TBRF is the public health problem in the region.

The study revealed that more than 62% of the householders keep chickens indoors at night and 35.5% of them reported to keep pigs in the pigpens adjoining to their households. This habit of keeping animals proximate to the human habitations possibly creates high risks of domestic infestations as chickens and pigs have been shown to be both alternative hosts for ticks and reservoirs of *Borrelia spp* (Motshegwa, 2004). It has been also reported that keeping animals indoors encourages domestic tick infestations (Walton, 1964).

The study also showed that 85.4% of respondents reported that high domestic tick infestation occurs during dry seasons and 12.1% reported that infestations occur during wet seasons. This shows that domestic tick infestation occurs throughout the year with different infestation rates at different seasons. The result corresponded to the study conducted in 1964 in some parts of East Africa where Walton (1964) reported that *O. moubata spp.* occurs in dwellings all year round in intimate contact with the occupants, and transmission of spirochaetes is continuous and there are no sharp seasonal fluctuations.

Treatment-seeking behaviour due to TBRF was related to cultural beliefs about the cause and cure of illness (Oberlander *et al.,* 2000; Makundi *et al.,* 2006). The results showed that majority (84.3%) of respondents sought medical treatment from the local health facilities. Only 2% sought medical services from traditional healers, 12.1% of respondents reported to have used self-medication at home either using traditional medicines (9.1%) or medicines from medical stores/shops (3%). Our study showed that the main health-seeking behaviour was to consult the nearest health facility (Makulu dispensary, 5km from Muungano village) or health personnel together with using

traditional medicines or herbs. Commonly people started care for a febrile child at home with what was available (herbs, remaining drugs, drugs from shops, tepid sponging), when there is no response or if the condition deteriorates then they sought advice from health personnel or go to the near health facilities. Few people sought medical treatment from traditional healers possibly because they had no money to meet the expected cost of treatment at health facility. This shows that some people get incorrect medical treatment for TBRF related illnesses. Advocacy is needed to change people's health seeking behaviour from self-medication to early diagnosis and prompt treatment from health facilities. However, self-treatment and traditional medicine are habitual among the population in Tanzania that calls for behavioural change intervention. Similar findings have been reported in other parts of Tanzania (Tarimo, *et al.,* 2000, Makundi *et al.,* 2006).

Control measures mentioned to be used against ticks in the study site were divided into physical and chemical methods. Physical methods included short term measures such as sprinkling of hot water onto floors and walls to kill the ticks, and mixing water and earth to plaster the surfaces of walls and floor to render them smooth and eliminate the cracks where ticks prefer to hide. However, lack of water in Dodoma region was a major constraint for sustaining these practices.

Chemical measures involved acaricides of the synthetic pyrethroid group. Locally used insecticides that have found to be active against ticks were lambdacyhalothrin (ICON), permethrin and deltamethrin. Some householders have been using the deltamethrin, supplied in net treatment kits, diluted with water, to sprinkle on the floor and walls. However, the inadequate concentrations used by the community for this purpose may lead to the emergence of tick resistance to the insecticide. These methods were not effective as domestic tick infestations continued to exist. The long-term solution would be to make floors and walls solid with cement mixtures and to use corrugated iron roofing instead of traditional 'tembe' wood and earth construction. However, many families are unable to afford the costs of cement and corrugated iron building materials. Some also prefer traditional roofs that are cooler in hot dry weather.

In conclusion, TBRF has been perceived as a major public health problem in the region and majority of people in the area know how TBRF is transmitted. Poverty and poor housing in the area are the major cause of domestic tick infestations and consequently, TBRF transmission. Thus, improving housekeeping and living standards of people and literacy to at least primary school level, coupled with health awareness campaigns, has the potential to reduce TBRF transmission. However, these calls for designing appropriate and sustainable community based- intervention for control of domestic tick infestations and prevent TBRF transmission in the region.

CHAPTER 4

INSECTICIDE TREATED BEDNETS FOR VECTOR CONTROL OF TICK BORNE RELAPSING FEVER: RANDOMISED CONTROLLED TRIAL

4.1 INTRODUCTION

Although the true burden of TBRF in Africa remains unknown, it is clear that in affected communities, TBRF is a serious cause of morbidity and mortality, and a challenge to public health that demands attention. Improving house-keeping and construction of permanent concrete buildings has long been believed to reduce domestic *Ornithodoros moubata s.l.* infestations (Walton, 1964). But given the socioeconomic status of many afflicted communities, this is not feasible. However, as domestic *O. moubata s.l.* infestations can be high, indoor residual spraying with insecticides has always been considered suitable as one of control measures. Early workers proposed the use of gammexane as a residual for persistent control (Knowles, 1950), and Talbert *et al* (1998) showed that pyrethroids could effectively clear houses of *O. moubata s.l.* for a number of years. Trials for control of soft and hard ticks using *Bacillus thuringiensis (Bti)* were conducted in Egypt in 1997 and showed that *Bti* may be effective against *O. moubata s.l.*, but more studies are needed (Hassnain *et al.,* 1997).

Applying residual insecticide to fabrics to prevent vector-borne diseases such as malaria and leishmaniasis began during World War II when the Soviet, German, and US forces used insecticide impregnated nets and clothing to protect from malaria (Curtis *et al.,* 1991). ITNs or curtains can have profound impact on malaria transmission by reduction of various entomological indices (Lindsay *et al.,* 1989; Lengeler & Cattani, 1996; Mbogo *et al.,* 1996) and more importantly, by diminution of morbidity and mortality in humans (Magesa *et al.,* 2005). ITNs have also proven to be effective against leishmaniasis (Kroeger *et al.,* 2002), filariasis (Pedersen & Mukoko, 2002) and Chaga's disease in South America (Kroeger *et al.,* 2003). The studies conducted in Venezuela and Colombia showed that ITNs are effective in protecting people from bites from

Phlobotoms (sand flies) and *Rhodnius* (Triatomine) vectors of leishmaniasis and Chaga's diseases respectively. Insecticide-treated bed-nets (ITNs) are distributed widely and their use against malaria is heavily promoted across Africa. ITN information, promotion, distribution and sales are particularly high and widespread in Tanzania (Lengeler, 2004; Magesa *et al.*, 2005).

ITNs are highly effective in preventing transmission of nocturnally transmitted vector-borne diseases, including non-flying vectors: Triatomine bug vectors of Chaga's disease in South America (Kroeger *et al.*, 2003). However, the efficacy of ITNs on vector control of TBRF has never been assessed. We considered whether pyrethroid insecticide treated bed-nets might also be effective against domestic tick populations and prevent transmission of TBRF. Given that many of the rural population in affected areas in central and western Tanzania (and presumably elsewhere) do not possess raised beds and typically sleep on matting blankets or animal hides on the floor, it would not be possible for ITNs to be tucked in neatly, as bed users are often advised to do, to form a closed barrier around the sleeper. However, we assumed that a loosely hung net in low ceiling huts would gather in bundles on the floor around the sleeper, and thus form a suitable barrier to crawling *O. moubata s.l.*

4.1.1 Objectives

The main objective of the study was to evaluate the efficacy of ITNs for vector control of tick-borne relapsing fever in Tanzania. Specifically, the trial wanted to determine whether or not insecticide treated bed nets could:

4.1.1.1 reduce or eliminate domestic *O. moubata s.l.* infestations,

4.1.1.2 reduce tick biting in treated households

4.1.1.3 reduce transmission of *Borrelia duttonii* to humans.

Outcomes were measured by a range of vectorial parameters, *Borrelia* incidence and the community's acceptance of the intervention for this purpose.

4.2 MATERIALS AND METHODS

4.2.1 Study site

The study was conducted in Muungano village (6°37'0S, 37°16'0E) which is located approximately 12km Northern-east of Mvumi hospital, 40km South East of the Tanzanian state capital, Dodoma (figure 1.5.3b). It is one of 128 villages in Dodoma Rural District (6° 30' to 8° 0'S, 35° 30' to 37° 0'E). The village has a total population of 7,500 people, distributed in 15 hamlets (known as 'vitongoji') with 1,667 households (United Republic of Tanzania, National Census, 2002). Muungano village was selected for the trial as it had no history of ITN usage or of any major anti-tick measures being undertaken. Meetings with community leaders were positive and the population were interested and willing to accept the trial. Full descriptions of the study site are presented in section 1.5.3.

4.2.2 Study population and sample size determination

The sample size for ITNs trial was determined based on a 5.3% prevalence of *Borrelia* transmission in children under five years old at baseline survey in the study site (Kisinza *et a.,* 2003), and on a 67% reduction of TBRF admissions in the paediatric ward at Mvumi hospital following the introduction of ITNs in 1996 for prevention of malaria in the region (Hospital records: *unpublished*).

An EpiInfo v6.4 software (CDC, Atlanta, GA, USA) was used to calculate a sample size of at least 1,472 households (736 in the treated and 736 in the control households) at a confidence level of 95% (i.e. probability that if two SAMPLES differ this reflects a true difference in the two POPULATIONS) with a power of 90% to detect a reduction of at least 57% of *Borrelia* infections in the treated households (i.e. reduction of *Borrelia* transmission from 5.3% to at least 2% in the treated households). To allow for 6% loss to follow-ups, 44 households per group was added to make a total sample size of 1,560 households (780 in the treated and 780 in the control households). The

principle of sample size calculation was based on a standard formula of sample size determination (Riffenburgh, 1999): $n = [(Z_1 - \alpha/2 + Z_1 - \beta/)^2 \delta^2]/d^2$ (where: n = sample size; $(Z_1 - \alpha)$ = level of significance (95% CI); $(Z_1 - \beta)$ = Power of detection (90%); δ^2 = Standard deviation, d = difference in sample proportions).

Table 4.2.2: Summary of sample size determination of the trial

Confidence level	Power	Exposed : unexposed Ratio	Disease in Exposed	Risk Ratio	Odd Ratios	Sample sizes + (6% x n)		
95%	90%	1 : 1	5.3%	2.65	2.75	Exposed	Unexposed	Total
						780	780	1,560

4.2.3 Study design

A randomised controlled trial (RCT) of insecticide treated bed-nets for control of domestic *O. moubata s.l.* infestations and prevention of TBRF was conducted between January 2003 and April 2005. It was intended initially to collect data over a two-year period to allow monitoring of any potential decrease in TBRF and tick population in the treated groups, and to monitor seasonal pattern and annual fluctuations in incidence, but as the result of a number of factors, it became necessary to extend the trial for an additional period of 5 months (see below).

Individual households were randomised to either receive insecticide-treated bed-nets, or to act as control. No matching of households in either arm was made. All traditional style constructed 'tembe' houses (rectangular with a flat roof of sod or earth supported by poles and walls of mud plastered wicker or sun-died bricks) were eligible for inclusion in the trial. But all households whose floors and walls were plastered with cement and roofed with corrugated iron sheets were excluded from the survey because it has long been known that they are rarely if ever infested with *O. moubata s.l* (Walton, 1957; Talbert *et al.*, 1998).

A two-stage random sampling technique was used to select the households for the trial. All households in the village were first listed and assigned numbers, and then randomised to get a total of 1,560 households for the trial, which were further randomised to allocate a total of 780 households to either receive insecticide-treated bed-nets, or act as control. Randomisation and allocation of households to either receive insecticide-treated bed-nets, or to act as control were conducted in front of both village leaders and heads of households. At the end of the trial, all control households were provided with ITNs.

The effects of ITNs were monitored over 29 months period by biannual surveys both during rainy (April) and dry (August) seasons. The effects of the trial on vector control of TBRF transmission were measured using both vectorial data (domestic tick infestations, densities and tick-feeding success) and *Borrelia* infection data. The primary outcome measures were therefore: percentages of households infested with *O. moubata s.l.* (domestic tick infestations), numbers of *O. moubata s.l.* per house (tick-density) and relative abundance of different developmental tick stages per house. The secondary outcome measures were the levels of *Borrelia spp.* infections in under five children as determined by thick blood smear (BS) and PCR techniques.

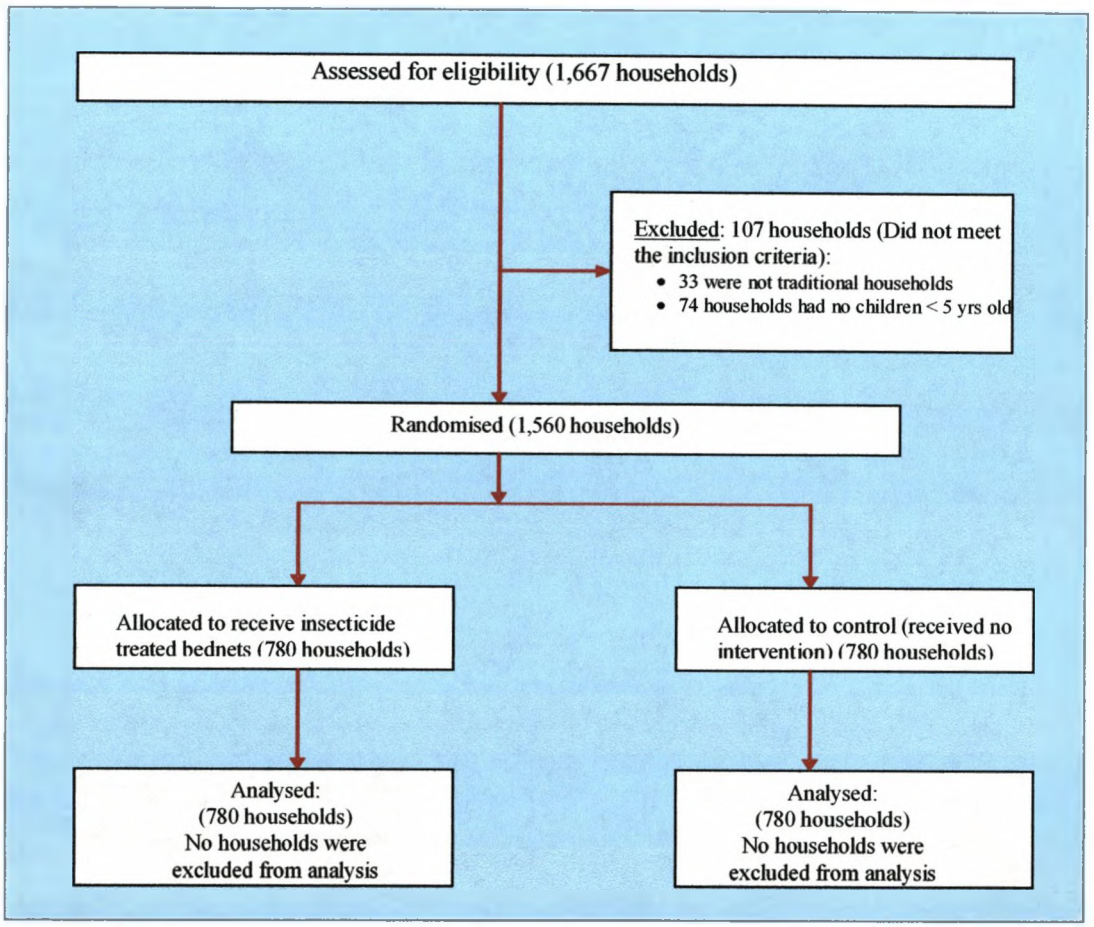


Figure 4.2.4: Flow chart of households to the trial

Based on the 88% prevalence of domestic tick infestations reported in some villages in Mvumi area central Tanzania (Talbert *et al.,* 1998), an EpiInfo v6.4 software (CDC, Atlanta, GA, USA) was used to calculate a sample size of at least 400 households (200 in the treated households and 200 in the control households) at a confidence level of 95% with a power of 90% to detect a reduction of at least 15% domestic tick infestation in the treated households (i.e. reduction of infestation from 88% to at least 75% in the treated households).

To determine the effects of ITN on domestic *O. moubata s.l.* infestations and densities, cross-sectional surveys from 400 households randomly selected (200 in the treated group and 200 in the control group) were conducted twice per year both during wet and dry seasons (April and August respectively) over 29 months period. The effects of the intervention on *Borrelia* infections were determined by screening blood samples from under five children in 1,560 households (780 in the treated group and 780 in the control group).

To avoid dilution effect of the intervention from *Borrelia* infections in the same household, only one child under five years old was randomly selected from each household and screened for *Borrelia* infections. In very rare instances due to ethical reasons, we screened all children or when a symptomatic child requested for the services at the same household, but only one child that was randomly selected included in the study and others just received the requested services. Both vector and *Borrelia* infection surveys were conducted concurrently reducing the number of visits to every household to a minimum. The surveys were carried out at baseline (October 2002) and then during follow-ups in April and August 2003; April and August 2004, and in April 2005 after the trial began in January 2003.

4.2.4 Intervention tools

Both lambdacyhalothrin (ICONET®) and bed nets were donated to the TBRF project by Syngenta (Syngenta Crop Protection AG, Basel, Switzerland). In total, 2,500 green round-shaped medium size bed-nets (1.5m wide, 1.8 m long and 2.1 m high with a total area of 2.7 m^2) made of 75-denier polyester fibre (Tanzania manufacturing Textile Limited factory) were donated. The research team impregnated all the bed nets with lambdacyhalothrin (ICONET®) insecticides (at the recommended dosage of 55.6mg/m^2 for mosquito control) at Mvumi hospital in January 2003 before distributed to the householders in the treated arm in Muungano village.

4.2.5 Susceptibility tests of *O. moubata s.l.* to lambdacyhalothrin treated netting

To determine baseline susceptibility of *O. moubata s.l.* to lambdacyhalothrin treated netting, bioassay tests were conducted in the laboratory at Mvumi hospital in December 2002. Two nets were treated with synthetic lambdacyhalothrin and the other two untreated bed nets were used as control.

The WHO standard bioassay procedures for mosquitoes (WHO, 2004) were modified for ticks. Briefly, the bases of WHO plastic cones were wrapped with a piece of bed net (15 cm x 15 cm) treated with lambdacyhalothrin at a dosage of 55.6mg/m^2 and unfed ticks (collected from households with no history of insecticide use in Muungano village) were introduced through the cone aperture, dropping onto the netting (See figure 4.2.5a). Four tick-developmental stages were each tested at three periods of exposure (15, 30 and 60 minutes). A total of 10 unfed ticks from each developmental stage were used in each WHO plastic cone at each period of exposure. The bioassay tests were conducted at ambient room conditions (27 ± 2°C and 65 – 70% RH). Each set of bioassays comprised 12 WHO test plastic cones with 120 ticks for treated nets. At the same conditions, the procedure was repeated with control netting (untreated). After exposure, ticks were kept in petri dishes and mortality observed after 24 hours (Figure 4.2.5b).

Figure 4.2.5 (a): WHO Bioassay plastic cones wrapped with netting in position, and ticks being introduced through the core top.

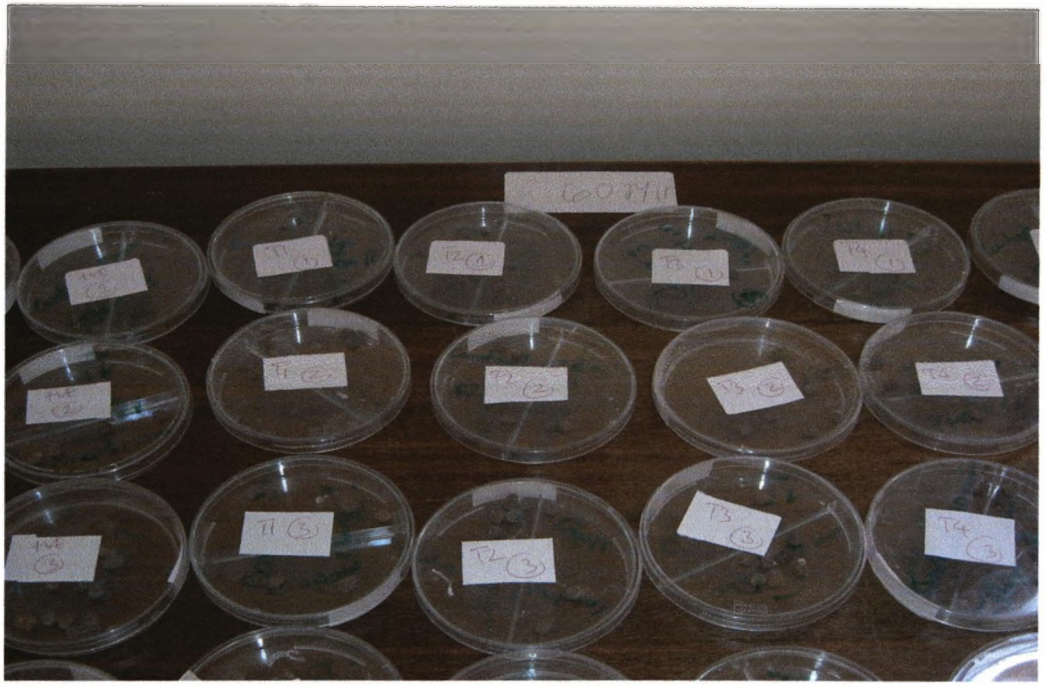


Figure 4.2.5(b): Ticks in petri dishes after exposure in netting

4.2.6 Distribution of insecticide-treated bed nets

The ITNs were then distributed to the 780 households in the treatment arm in January 2003, at an average of 3 nets per household, sufficient to ensure that all children in each household slept under a treated net (one net for parents, the second to the female children and the other for male children)(See figure 4.2.6a).

Householders provided with ITNs were instructed on correct practice for use and safety and informed on the need to re-impregnate the nets (Figure 4.2.6b). To ensure that all nets were re-impregnated properly with insecticide at the right time, householders were instructed to bring their nets to the village office for re-impregnation under the supervision of the research team, and requested not to re-treat the nets with any insecticide at any other time, during the study (See figure 4.2.6c).

All insecticide treated bed-nets were re-treated twice annually: The first and second treatments were conducted in January and July 2003, six months later. The third and fourth re-impregnations were conducted in July 2004 (12 months interval[8]) and February 2005 (6 month) respectively. There was a delay of an additional 6 months re-impregnation between July 2003 and July 2004 because the insecticides could not be obtained in time from Syngenta.

The nets brought at each re-treatment session were first counterchecked with numbers allocated to each household and if found missing, the head of the household was questioned on the whereabouts of missing nets. The missing nets were replaced with new treated ones immediately. However, only two cases were reported as missing nets throughout the trial.

[8] An overdue of 6 months of re-impregnation of nets as insecticides were received late from Syngeta (Basel, Switzerland)

Figure 4.2.6 (a) Registration and distribution of insecticide treated bed nets to the householders in the treated group in Muungano village, January 2003

Figure 4.2.6(b): Demonstrations on how to use and tuck ITNs on animal hides in Muungano village, January 2003

Figure 4.2.6 (c): Re-impregnation of bed nets in the village, July 2003

4.2.7 Effects of ITNs on domestic tick infestations and densities

A baseline survey on domestic tick infestations, distribution within the houses and densities were conducted between October and November 2002 in the study village before ITN trial began. Surveys on the effects of ITN trial on domestic infestations and densities were conducted at 5 cycles (April and August 2003, April and August 2004 and April 2005) between January 2003 and April 2005.

The surveys were conducted in 400 "tembe" households (200 in the treated group and 200 in the control group) randomly selected from the village. Detailed descriptions of the protocols/procedures for sampling ticks are presented in section 2.2.2 (See figure 4.2.7 A, B &C). To avoid psychological bias, the research team collected ticks from the selected households blindly (without knowing whether was treated or control household) until when the surveys were accomplished.

Figure 4.2.7: (A) Sampling of domestic tick population in the households (B) collecting ticks from the floor and (C) sieving the soil substrates to collect early tick stages

4.2.8 Effects of ITNs on tick feeding success

For insecticide treated bed nets to be effective, they must either provide physical barriers to man-vector contact or repel ticks from feeding on human hosts. A field experiment was conducted in January 2004 in Muungano village to determine if tick population in the treated households were able to obtain blood or fed on people sleeping under treated bed nets

Before field experiments were conducted, informed consent was first obtained from both the village leaders and the heads of households and a total of 20 households (10 in the control group and 10 in the treated group) were randomly selected. Only households without animals such as dogs, cattle or pigs were selected and members of the households were told not to keep animals or chickens indoors throughout the field experiment.

To make sure that neither animals nor chickens were kept indoors in the selected households, follow-ups were conducted for 7 days consecutively before carrying out a collection of ticks in the households. Ticks were collected from four sites in each household (bedroom, seating areas, kitchen, and poultry areas) as described in section 2.2.2. Sorting and grading of all ticks into their respective developmental stages were conducted in the laboratory at Mvumi Hospital. Detection of fresh blood-fed ticks was conducted by squashing individual ticks onto Whatman® filter papers (3M, Maidstone, UK).

4.2.9 Prevalence of *Borrelia* infection in children under five years old at baseline

A baseline survey was conducted between October and November 2002 to establish the prevalence of *Borrelia* infection in children under five years old. Detection of *Borrelia* infection was done by taking finger-prick blood samples from 361 under five children (54 symptomatic and 307 asymptomatic children). A household survey was first conducted in the village to detect all symptomatic/febrile children (auxiliary

temperature >37°C associated with vomiting, feeling cold, joint/ back pain or headache) and one asymptomatic children was selected from each household surveyed and detection of *Borrelia* infections was determined by thick blood smear (BS) and PCR.

Blood smears

Thick blood smears were prepared and dried at the village before staining with Giemsa and microscopic examination in the laboratory at Mvumi hospital. All individuals found to have *Borrelia* spp infections were treated with PPF (procaine penicillin fortified with iron, 120 mg intramuscular injection daily for 5 days) and followed up for 7 further days. Individuals with *Plasmodium* species infections were informed and directed to the nearest dispensary (Mvumi Makulu, 5 km from Muungano village) for treatment. All infections were susceptible to antibiotic treatment (no treatment failure reported).

Figure 4.2.9: A medical laboratory technician taking finger prick-blood samples for thick blood smears at Muungano village at baseline survey in October 2002

PCR examination of samples

Blood from the same individuals was also spotted and dried onto small pieces of filter paper (approximately 5 mm diameter; Whatman 3M, Maidstone, UK), put in labeled Eppendorf tubes with pierced lids, and stored in sealed plastic bags containing silica gel. PCR sequencing and subsequent analyses were carried out by staff in the laboratory of Professor Masahito Fukunaga (Laboratory of Molecular Microbiology, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima, Japan. Full details of procedures are given in Fukunaga *et al* (2001). Briefly, the PCR is based on the highly conserved gene has been shown to be suitable for both detection and classification of the *Borrelia* genus (Fukunaga *et al.*, 2001). Positive samples were directly sequenced in both directions with an ABI3100 automated sequencer (Perkin Elmer, Boston, USA) to identify the *Borrelia* species present. A phylogenetic tree was constructed using the DNASTAR and CLUSTAL W software packages, using published sequence data to classify the types of *Borrelia* species in the study site.

4.2.10 Effects of ITNs on prevalence of *Borrelia* infection in children under five years old

Surveys for *Borrelia* infections were conducted at 5 cycles [(April and August 2003 (3 and 7 months post intervention respectively), April and August 2004 and April 2005 (15, 18 and 25 months post intervention respectively)] from 1,560 children under five years old (780 in the treated group and 780 in the control group) on each occasion selected randomly. The same protocols used in the baseline survey were also deployed here. In order to compare the *Borrelia* infection rates prior and post ITNs trial, blood samples from baseline surveys were re-analyzed by staff in the laboratory at Liverpool School of Tropical Medicine using the same PCR technique (Fukunaga *et al.*, 2001) as used during baseline survey on *Borrelia* infections.

4.2.11 Acceptance of ITNs intervention

A study on knowledge, attitudes and perceptions was conducted in April 2004 (14 months after the ITN trial began) in the village to determine the community's acceptance and perception of ITN efficacy to control domestic tick infestations and TBRF transmission. A structured questionnaire was designed and household interviews were conducted to a total of 409 heads of households (210 in the control group and 199 in the treated group) randomly selected from the village. People were asked on the domestic tick infestations and tick-bites, and if there was any perceived efficacy of ITNs on TBRF-vector control in Muungano village.

4.2.12 Assessment on the use of ITNs and other anti-tick activities in the study site

Household interviews were conducted in April 2005 (at the end of the trial) to assess the use of ITNs and other anti-tick activities in the study site. A structured questionnaire was designed and a total of 300 heads of households were randomly selected (150 in the treated and 150 in the control households) and interviewed.

Questions asked in the household survey included use of insecticide treated bed nets, sleeping behaviour and use of insecticides other than ITNs. People were also inquired on the use of other anti-tick activities. Additionally, heads of households in the treated group were inquired on the irregular use of bed nets (whether there were times or seasons of the year when the nets were not used such as when there were more visitors in the households, during hot seasons, when there were no ticks or insects seen or any other reasons). The full questionnaire is shown in appendix 4.2.12.

4.2.13 Effects of net washing on ITN efficacy

A study was conducted in April 2003 (3 months after ITN trial began) in Muungano village to (1) find out how often the nets were washed and (2) determine the effects of washing on ITN efficacy against *O. moubata s.l.*

Frequency of net washing

To determine how often the nets were washed in the study site, a total of 209 households were randomly selected from the treated households. Heads of the selected households were interviewed on how often their nets were washed. Also they were inquired if they had re-treated the nets. Frequencies of net washing were clustered according to how frequently they had been washed and one net from each cluster was randomly selected and taken for bioassay tests in the laboratory at Mvumi hospital. A replacement of new treated bed net was provided immediately.

Bioassays

The same protocol described in section 4.2.5 was used again here. Bioassay tests were conducted on netting removed from the lower sides of each individual treated bed net. Bioassays were run parallel with a positive control (a freshly impregnated bed net) and negative control (with untreated bed net). Two replicates were conducted in each test. After exposure, ticks were kept in Petri-dishes and mortality observed after 24 hours.

4.2.14 Statistical Analyses

Data entry and check files for the community interviews, tick infestations and *Borrelia* infections were prepared in Epi-Info version 6.04, and then transferred to STATA statistical analysis software package version 7 (Stata Corp., College Station, TX, USA, 2003) for further analysis.

The effects of ITNs were determined by comparing the outcome measures of the trial (described in details in section: 4.2.3) between control and treated households. χ^2 tests or in case of small numbers, Fisher's exact tests were used to test differences for categorical variables. Poisson regression tests were used to test differences in the mean number of ticks between control and treated households at each sampling period.

4.3 RESULTS

4.3.1 Prevalence of domestic *O. moubata s.l.* infestations at baseline

Prevalence of domestic tick infestations at baseline survey in the study site ranged from 50% to 87.5% (See figure 4.3.1) with a mean tick infestation rate of 71.5 ± 10.9% (SD). The number of households infested with ticks in the village were homogeneously distributed between hamlets, and domestic tick infestation rates did not differ significantly between hamlets (Fisher's exact test: $p = 0.836$). The mean number of ticks per infested household was 4.91±2.39 ticks (SD) (table 4.3.1). Tick developmental stage distribution differed significantly between hamlets (Fisher's exact test: $p < 0.001$).

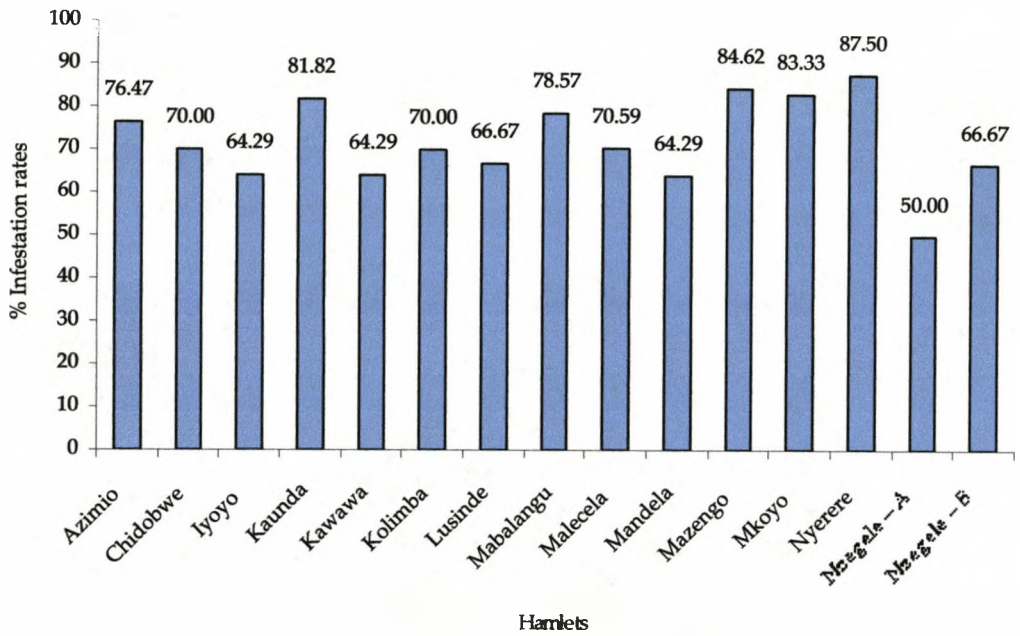


Figure 4.3.1 Domestic tick infestations in Muungano village shown by hamlet ('vitongoji'[9])

[9] small settlement with local boundaries within the village

Table 4.3.1: Domestic tick infestations at baseline survey conducted between October and November 2002 in Muungano village shown by hamlets

Hamlets	No. of households surveyed	No. of households infested	% infestations	Tick-stages				No. of ticks in infested households	Mean No. of ticks per household	± SD
				Stage-1	Stage-2	Stage-3	Stage-4			
Azimio	17	13	76.5	17	22	20	13	72	4.24	2.065
Chidobwe	10	7	70.0	5	8	28	5	46	4.60	2.240
Iyoyo	14	9	64.3	20	6	13	9	48	3.43	1.670
Kaunda	11	9	81.8	16	5	8	5	34	3.09	1.505
Kawawa	14	9	64.3	6	8	13	15	42	3.00	1.461
Kolimba	10	7	70.0	0	1	16	5	22	2.20	1.071
Lusinde	15	10	66.7	4	8	19	17	48	3.20	1.558
Mabalangu	14	11	78.6	20	28	38	27	113	8.07	3.930
Malecela	17	12	70.6	36	28	28	19	111	6.53	3.180
Mandela	14	9	64.3	4	15	15	8	42	3.00	1.461
Mazengo	13	11	84.6	34	68	33	10	145	11.15	5.430
Mkoyo	6	5	83.3	21	10	10	0	41	6.83	3.326
Nyerere	16	14	87.5	16	17	27	16	76	4.75	2.313
Nzegele – A	14	7	50.0	34	14	19	8	75	5.36	2.610
Nzegele – B	15	10	66.7	7	22	26	8	63	4.20	2.045
Total	200	143	71.5	255	260	313	165	993	4.91	2.391

4.3.2 Susceptibility of *O. moubata s.l.* to lambdacyhalothrin treated netting

Baseline bioassay results showed that *O. moubata s.l.* was highly susceptible to lambdacyhalothrin treated netting. Mortality rates ranged from 86.25% (69/80) to 97.5% (78/80) of all ticks exposed to the insecticide at 15 and 60 minutes respectively (Figure 4.3.2). All tick stages were equally susceptible, mortality rates between developmental tick stages ranged from 85% to 90% at a mean mortality rate of 86.25±0.5 (SD). Full data are presented in table 4.3.2

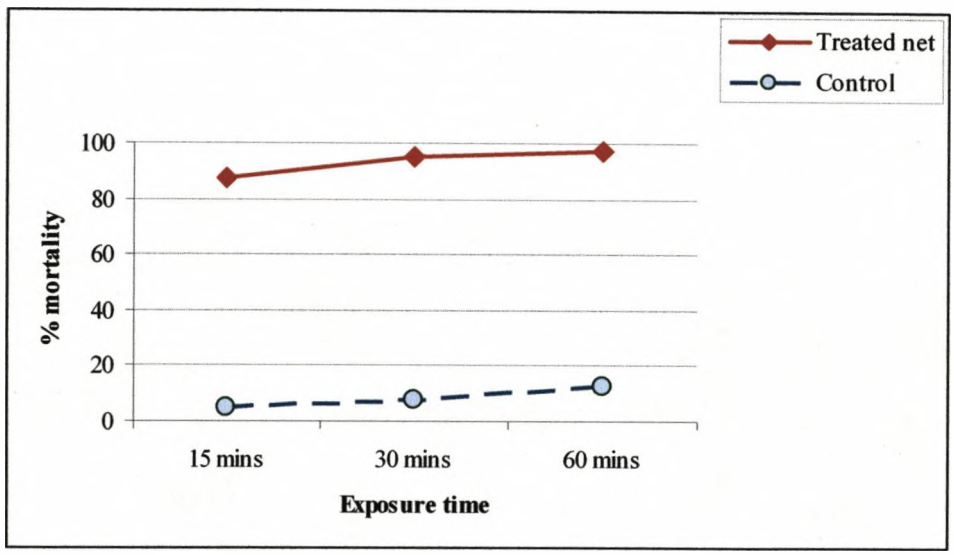


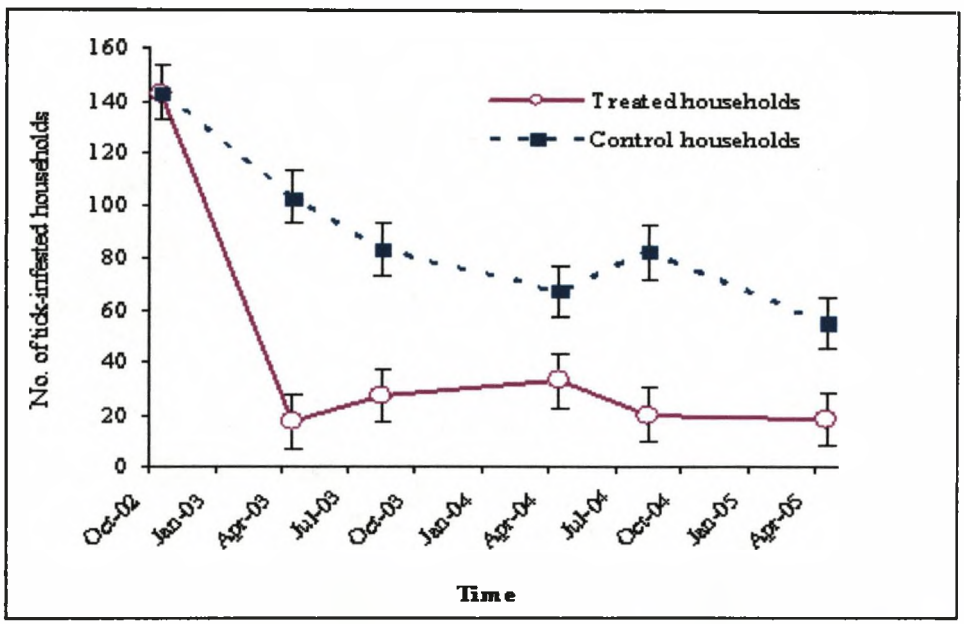
Figure 4.3.2: Mortality rates of *O. moubata s.l.* at different time of exposure to lambdacyhalothrin treated netting in the laboratory at Mvumi hospital

Table 4.3.2: Mortality rates of *O. moubata s.l.* at different time of exposure to lambdacyhalothrin treated netting

Tick-stages	Treated netting			Control		
	15 mins	*30 mins*	*60 mins*	*15 mins*	*30 mins*	*60 mins*
Stage-1	85% (17/20)	95% (19/20)	95%(19/20)	0% (0/10)	0% (0/10)	30% (3/10)
Stage-2	90% (18/20)	95% (19/20)	100% (20/20)	10% (1/10)	0% (0/10)	10% (1/10)
Stage-3	85% (17/20)	95% (19/20)	95% (19/20)	0% (0/10)	20% (2/10)	0% (0/10)
Stage-4	85% (17/20)	90% (18/80)	100% (20/20)	10% (1/10)	10% (1/10)	10% (1/10)
Total	86.25% (69/80)	93.75% (75/80)	97.5% (78/80)	5% (2/40)	7.5% (3/40)	12.5% (5/40)

4.3.3 Effects of ITNs on domestic *O. moubata s.l.* infestations

Domestic tick infestation rate in the control households remained higher (51.5%; 103/200) than in the treated households (8.5%; 17/200) in April 2003 ($p < 0.001$). The relative risk ratio of domestic tick infestations in the control households was six times more than in the treated households in April 2003 after which the relative risk of tick infestations steadily declined over time (see figure 4.3.3). Domestic tick infestation rates in the control households remained significantly higher than in the treated households throughout the trial (Poisson regression test: $p < 0.001$). The results also showed that domestic tick infestation rates in the treated households was significantly lower than at baseline survey (Fisher's exact tests: $p < 0.001$) (See table 4.3.3).



[10]Figure 4.3.3: Domestic tick infestations between treated and control households [treated nets were distributed in January 2003; and re-impregnation conducted in July 2003, July 2004[11] (12 months after the first re-impregnation) and February 2005]

[10] Bars showing ± standard errors of mean (SEM) (i.e. index of reliability that expresses variation between observers and other causes of differences between repeated observations)

[11] An overdue of 6 months reimpregnation of nets as insecticides were received late from Syngeta (Basel, Switzerland)

Table 4.3.3: Domestic tick infestations in treated and control households in Muungano village between 2002 and 2005

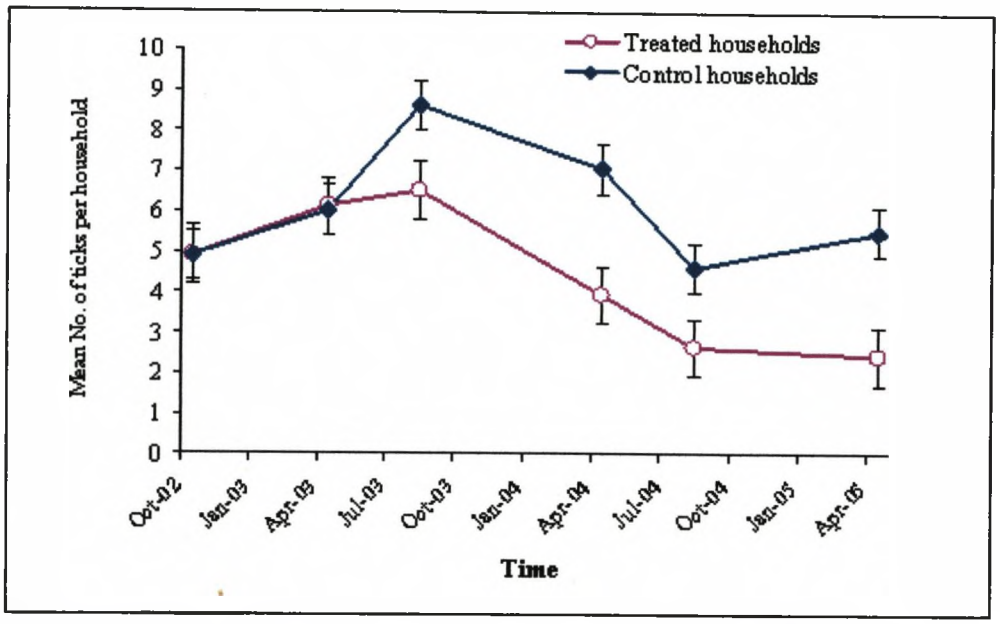
	Treated (N = 200)		Control (N =200)		Test Statistics *(ITNs Vs Control)*		
	No. infested	*% infestations*	*No. infested*	*% infestations*	*95% CI*	*RR*[12]	*P- value*
Oct-02[13]	143	71.50	143	71.50	-	-	-
Apr-03	17	8.50	103	51.50	3.82 - 9.76	6.06	0.001
Aug-03	27	13.50	83	41.50	2.10 - 4.54	3.07	0.001
Apr-04	43	21.50	67	33.50	1.13 - 2.17	1.56	0.01
Aug-04	20	10.00	82	41.00	2.65 - 6.43	4.10	0.001
Apr-05	18	9.00	39	19.50	1.30 - 3.65	2.17	0.004

4.3.4 Effects of ITNs on domestic tick population density

The mean numbers of ticks per household in the treated households were significantly lower than in the control households (Poisson regression test: $p < 0.001$) except in April 2003 when the mean numbers of ticks per household between control and treated households were not significantly different (Poisson regression test: $p = 0.867$) (Figure 4.3.4). Incidence rate ratios (IRR[14]) of domestic tick population in the control households increased significantly and steadily over the study period (see table 4.3.4).

The domestic tick population density (total number of ticks per households) in the control households ranged from 5 to 7 ticks per household with a mean number per household of 6.46 ±1.39 (SD) ticks. Domestic tick population in the treated households ranged from 2 to 7 with a mean number of ticks per house of 4.77 ±2.01 ticks (SD).

[12] Relative Risk Ratio [(Incidence among exposed to risk)/Incidence among non-exposed to risk)]
[13] Domestic tick infestations at baseline survey in the study site between October and November 2002
[14] Incidence Rate Ratio [(mean number of ticks per control household)/(mean number of ticks per treated household)]



[15]Figure 4.3.4: Effects of ITNs on domestic tick population density in the treated households compared to control households between 2003 and 2005

[15] Bars showing ± standard errors of mean (SEM)

Table 4.3.4: Domestic tick population density between control and treated households in Muungano village (2002 and 2005)

	Treated households (N = 200)			Control Households (N = 200)			Test Statistics: (*Treated Vs Control*)		
	No. of households infested	*No. of ticks*	*Mean No. of ticks per house*	*No. of households infested*	*No. of ticks*	*Mean No. of ticks per house*	*95% CI*	*Incidence Rate Ratio (IRR[16])*	*p-values*
Oct-02[17]	143	993	4.91	143	993	4.91	-	-	-
Apr-03	17	104	6.12	103	619	6.01	0.798 - 1.209	0.982	P = 0.867
Aug-03	27	176	6.52	83	715	8.61	1.121 - 1.558	1.322	P < 0.001
Apr-04	43	170	3.95	67	472	7.04	1.495 - 2.123	1.782	P < 0.001
Aug-04	20	53	2.65	82	379	4.62	1.308 - 2.325	1.744	P < 0.001
Apr-05	18	44	2.44	39	215	5.51	1.631 - 3.119	2.255	P < 0.001

[16] IRR = (mean number of ticks per control household)/(mean number of tick per treated household)
[17] Domestic tick infestations at baseline survey in the study site between October and November 2002

4.3.5 Effects of ITNs on distribution patterns of ticks in the infested houses

Distribution patterns of *O. moubata s.l.* in the infested houses were assessed before and after the trial. The highest percentage of *O. moubata s.l.* at baseline (October 2002) was found in the bedrooms (75.9%) Other sites of the houses with lower percentages of tick distribution were the seating areas (13.2%), kitchen (3.8%) and poultry areas (7.1%) (See table 4.3.5a). The highest percentages of tick distribution during the intervention were also found in bedrooms ranged from 91.3% to 61.4% for April 2003 and April 2005 respectively. Other sites with low percentage distribution were seating areas (range: 8.7% to 27. %); Kitchen (Range: 0% to 6.8%) and poultry areas (range: 0% to 4.6%). In April 2003 (3 months after the trial began) the percentage distribution of ticks in the bedrooms showed a gradual and steady decline throughout the trial while percentage distribution of ticks in kitchens showed a steady increase from April 2003 to August 2004. The pattern of tick distribution in the poultry areas remained low throughout. There was no significant difference in the patters of the distribution of ticks within the infested houses between baseline and after the intervention ($p>0.05$) (see figure 4.3.5).

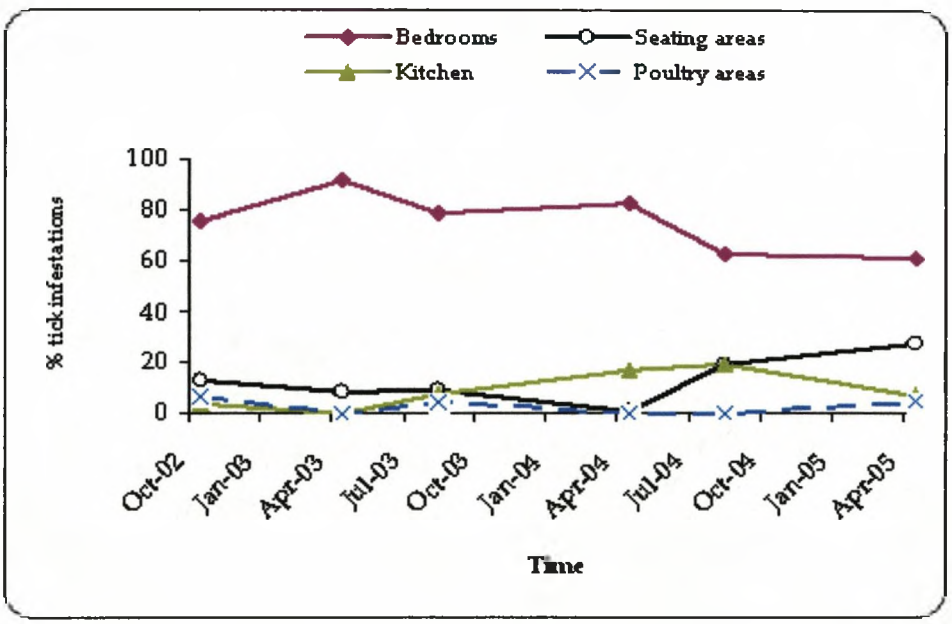


Figure 4.3.5: Distribution of ticks within infested houses at baseline (October 2002) and during ITNs intervention (between January 2003 and April 2005) in the treated households

Table 4.3.5 (a) Distribution of ticks within infested houses at baseline (October 2002) and during the trial (April 2003 – April 2005) in the treated households

	Total No. of ticks	Bedrooms		Seating areas		Kitchen		Poultry areas	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No*	*%*
Oct-02 (At baseline)	993	754	75.93	131	13.19	38	3.83	70	7.05
Apr-03	104	95	91.35	9	8.65	0	0	0	0
Aug-03	176	139	78.98	16	9.09	13	7.39	8	4.55
Apr-04	170	140	82.35	1	0.59	29	17.06	0	0
Aug-04	53	33	62.26	10	18.87	10	18.87	0	0
Apr-05	44	27	61.36	12	27.27	3	6.82	2	4.55

Table (b): Distribution of ticks within infested houses in the control households (April 2003 – April 2005)

	Total No. of ticks	Bedrooms		Seating areas		Kitchen		Poultry areas	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Apr-03	619	454	73.34	110	17.77	26	4.2	29	4.68
Aug-03	715	520	72.73	45	6.29	142	19.86	8	1.12
Apr-04	282	247	87.59	18	6.38	17	6.03	0	0
Aug-04	379	344	90.77	13	3.43	22	5.8	0	0
Apr-05	215	91	79.13	11	9.57	11	9.57	2	1.74

4.3.6 Effects of ITNs on tick feeding success

The effects of ITNs on ticks feeding success were assessed in January 2004 (one year post the trial began) in the treated households compared to control households. The results showed that 13.8% (20/145) of ticks in the treated households were detected with blood compared to 44.8% (65/145) in the control households. The difference was highly significant (Fisher's exact test: $p < 0.001$) (table 4.3.6).

The percentages of ticks with fresh blood meals in stages 2, 3 and 4 in the control households were significantly higher than in the treated households (Fisher's exact test: $p < 0.001$) but the difference in the percentages of ticks with blood meals in stage 1 between control and treated households was not significant ($p > 0.05$). Probably the small sized (first stage) ticks could get access to hosts by crawling under the nets.

Table 4.3.6: Percentages of domestic ticks detected with blood in control and treated households (January 2004): Analysis by Fisher's exact test

	Stage-1	Stage-2	Stage-3	Stage-4	Total
Control	15% (6/40)	61.8% (34/55)	47.2% (17/36)	57.1% (8/14)	44.8% (65/145)
Treated	8.7% (2/23)	6.3% (2/32)	25.5% (12/47)	9.3% (4/43)	13.8% (20/145)
RR	1.73	9.89	1.85	1.14	3.25
95% C.I	0.44 – 7.17	3.00 – 36.2	1.03 – 3.37	2.26 – 16.8	2.11 – 5.09
P -values	*0.741*	*0.0001*	*0.069*	*0.001*	*0.0001*

4.3.7 Prevalence of *Borrelia* infections in under five children at baseline[18]

Prevalence of *Borrelia* transmission in children under five years old was established during baseline surveys that was conducted in October – November 2002 prior ITNs trial in the study site. The results showed that prevalence of *Borrelia* infections in the children under five years old were 2.8% and 5.3% detected by microscopy and PCR respectively. Of the 54 children with fever (symptomatic children), three (6%) and six (11%) were found to have *Borrelia* sp infections by blood-slide and PCR, respectively (see table 4.3.7a). One individual's sample was positive by microscopy but not by PCR. Although the rate of malaria was high, only one individual was found to have both *Borrelia* sp (by PCR) and *Plasmodium* spp. Of the 307 randomly sampled afebrile asymptomatic children, six (2%) were positive by microscopy for *Borrelia* spp. *Borrelia* spp. was detected in 13 (4%) of these individuals by PCR, twice as many as with *Plasmodium* (by microscopy).

Table 4.3.7 (a): *Borrelia* species infections detected by Blood slide and PCR

	No. screened	Malaria[19]	TBRF	
		Blood slide	Blood slide	PCR
Symptomatic children	54	15 (27.8%)	3 (5.6%)	6 (11.1%)
Asymptomatic children	307	6 (2.0%)	7 (2.3%)	13 (4.2%)
Total	361	21 (5.82%)	10 (2.8%)	19 (5.3%)

Table 4.3.7 (b): *Borrelia* species infecting the community in the study site

	No. screened	Types of Borrelia species			
		B. duttonii type Ly	*B. duttonii* type 2(B)	Unknown type 3	Unknown type 5
Symptomatic children	54	4	1	1	0
Asymptomatic children	307	6	0	4	1
Total	361	10	1	5	1

[18] *Part of these data has been published as*: Kisinza, WN; McCall, P J; Mitani, H; Talbert, A; and Fukunaga, M. (2003) A newly identified Tick-Borne *Borrelia* species and Relapsing Fever in Tanzania. *THE LANCET, 362, 1283 -1284*

[19] Only *Plasmodium falciparum* was detected

4.3.8 Effects of ITN on prevalence of *Borrelia* spp. infection in children under five years old

Borrelia transmission in children under five years old were assessed between January 2003 and August 2005 in the study site. *Borrelia* infection was detected by both blood slide and PCR from the children in the treated and control households.

Detection of Borrelia infections by Thick Blood Smears

Prevalence of *Borrelia* infections detected by blood slide in April 2003 (four months after the trial began) was 0.26% (2/780) and 1.67% (1019) in treated and control households respectively (see table 4.3.8a). The relative risk of *Borrelia* infections in the control households was 6.5 times more than in the treated households and the difference was highly significant (Fisher's exact test: $p = 0.004$). No *Borrelia* infections were detected between August 2003 and April 2005 in the treated households. *Borrelia* infections in children under five years of age in the control households were detected only in April 2003 (17, 1.67%) and April 2004 (1, 0.19%).

The overall rates of *Borrelia* infections in control and treated households were 0.54% (18/3328) and 0.06% (2/3220) respectively over the entire trial period (table 4.3.8a). The relative risk of *Borrelia* infections in the control households was 8.71 times higher than in the treated households. Difference in incidence of *Borrelia* infections between control and treated households over the trial period was highly significant (Fisher's exact test: $p < 0.001$).

Malaria infection rates in children under five years old were 2.31% (77/3328) and 1.49% (48/3220) in the control and treated households respectively over the entire trial period (Fisher's exact test: $p < 0.05$) (see table 4.3.8b). The relative risk of malaria infection in the control households was 1.55 times higher than in the treated households. The difference in the incidence of malaria infections between the control and treated households during the trial period was significant (Fisher's exact test: $p < 0.05$).

Table 4.3.8 (a): Detection of *Borrelia* spp. infections by thick blood smears in under five years children (April 2003 – April 2005)

	Treated households			**Control households**			Test statistics (Fisher's exact test)		
	No. screened	*No. infections*	% infections	*No. screened*	*No. infections*	% infections	RR	95% CI	P-value
Apr-03	780	2	0.26	1019	17	1.67	6.51	1.68 - 25.3	0.004
Aug-03	628	0	0.00	600	0	0.00	Undefined[20]	N/A	N/A
Apr-04	637	0	0.00	517	1	0.19	Undefined	N/A	N/A
Aug-04	644	0	0.00	617	0	0.00	Undefined	N/A	N/A
Apr-05	531	0	0.00	575	0	0.00	Undefined	N/A	N/A
Total	3220	2	0.06	3328	18	0.54	8.71	2.25 - 33.7	0.001

Table 4.3.8 (b): Detection of *Plasmodium* spp. infections by thick blood smears in under five years children (April 2003 – April 2005)

	Treated households			**Control households**			Test statistics (Fisher's exact test)		
	No. screened	*No. infections*	% infections	*No. screened*	*No. infections*	% infections	RR	95% CI	P-value
Apr-03	780	19	2.44	1019	35	3.43	1.41	0.82 - 2.43	0.265
Aug-03	628	28	4.46	600	25	4.17	0.93	0.55 - 1.57	0.889
Apr-04	637	1	0.16	517	6	1.16	7.39	1.17 - 46.7	0.05
Aug-04	644	0	0.00	617	6	0.97	Undefined	N/A	N/A
Apr-05	531	0	0.00	575	5	0.87	Undefined	N/A	N/A
Total	3220	48	1.49	3328	77	2.31	1.55	1.09 - 2.21	0.019

[20] No analysis was done because number of infection was zero.

Detection of Borrelia spp. infections by PCR

Effect of ITNs on *Borrelia* transmission in children under five years old was also determined using PCR by screening blood samples obtained during the follow-ups. Because of financial constraints only 400 (6.11%) out of 6540 blood samples obtained at the end of the trial (April 2005) were screened for *Borrelia* infections. The rates of *Borrelia* species infections detected by PCR at the end of the trial in April 2005 were 10% (20/200) and 7.5% (15/200) in the control and treated households respectively (table 4.3.8c).

The relative risk of *Borrelia* infection in the control households was 1.33 times more than in the treated households, but the differences between control and treated households was not statistically significant (Fisher's exact test: p =0.480). Because of small number of blood samples screened for *Borrelia* infections [400 (6.11%)] presumably caused and underestimation of the effect of ITNs from prevention of *Borrelia* infections in the treated households.

Table 4.3.8 (c): *Borrelia* infections detected by PCR in April 2005 (24 months post intervention)

	No. screened	No. infected	% infections	Fisher's exact test		
				95% CI	RR	p-value
Control	200	20	10	0.71 – 2.51	1.33	0.480
Treated	200	15	7.5			
Total	400	35	8.75			

4.3.9 Acceptance of intervention

Socio-demographic characteristics of respondents

The mean age of respondents was 50 ± 13.27 years (SD) with a range of 25 to 70 years. The majority of respondents (54.28%, 222/409) had a primary school education while 45.72% (187/409) had not attended any formal education. The results also showed that there was an improvement on the possession of raised beds in the study site from 11% (22/198) at baseline in October 2002) to 22% (90/409) in April 2004 (14 months after the trial began) of which 14.2% (58/409) were in the control households and 7.8% (32/409) in the treated households (Fisher's exact test: $P < 0.01$).

Acceptance of ITNs in the study site was high: the majority of householders 88% (175/199) in the treated group reported that they had not seen domestic ticks neither other pests such as fleas and bedbugs as used to be seen before using ITNs (see table 4.3.9b).

Higher percentage of domestic tick infestations were reported in the control households (48.1%, 101/210) than in the treated households (12.1%, 24/199) and the difference was highly significant ($p<0.001$). Similarly, higher percentage of perceived tick-bites was reported in the control households (49.5%, 104/210) than in the treated households (7%, 14/199) and the difference between them was also highly significant (Pearson Chi-square test: $p < 0.001$) (see table 4.3.9a).

A higher proportion of people in the control households (47.1%; 99/210) used insecticides (other than ITNs) than in the treated households (23.6%; 47/199) (Fisher's exact test: $p < 0.05$). Thus, 14 months after intervention, 47.1% (99/120) of householders in the control group were using insecticides to control domestic tick infestations. This was investigated further in April 2005 (Section 4.3.10).

Table 4.3.9(a): Socio-demographic characteristics of respondents (April 2004)

Characteristics	Total		Control households (N = 210)		Treated households (N = 199)	
	Number	%	Number	%	Number	%
Mean age	50		51		49	
15 - 24	60	14.67	37	17.62	23	11.56
25 - 44	191	46.70	121	57.62	106	53.27
45 +	158	38.63	52	24.76	70	35.18
Sex Ratio (M: F)	149:260		80:130		69:130	
M	149	36.43	80	38.10	69	34.67
F	260	63.57	130	61.90	130	65.33
Education level						
Primary School	222	54.28	143	68.10	120	60.30
None	187	45.72	67	31.90	79	39.70
Occupation: All were peasants	409		210		199	
Use of raised beds						
Raised beds	90	22.00	58	27.62	32	16.08
None	319	78.00	152	72.38	167	83.92
Family size:						
Lower (< 4 people)	242	59.17	90	42.86	67	33.67
Higher (> 4people)	167	40.83	120	57.14	132	66.33

Table 4.3.9 (b): Percentage of perceived domestic tick infestations and tick-bites between control and treated households (April 2004)

	Control households (N=210)	Treated households (N=199)	Test Statistics		
			RR	*95% CI*	*P - values*
Tick-infestations	101 (48.1%)	24 (12.1%)	6.86	4.05 – 11.87	0.0001
Tick-bites	104 (49.5%)	14 (7.0%)	13.16	7.02 – 26.02	0.0001
'Tick-fevers'	17 (8.1%)	15 (7.5%)	1.09	0.50 – 2.42	0.8112
Insecticide use to control ticks	79 (37.8%)	37 (18.6%)	2.68	1.66 – 4.34	0.0001
Insecticide use to control domestic pests mosquitoes, fleas & bedbugs	20(4.9%)	10(4.8%)	1.89	0.87 – 4.15	0.154

4.3.10 Assessment on the use of ITNs and other anti-tick activities in the study site

In response to the observed reduction in ticks in control households during the study (see section 4.3.3), a cross-sectional survey was conducted in April 2005 (at the end of the trial) using household interviews to assess the level of anti- tick activities in the study site. A total of 300 heads or representative heads of the households (150 in the control and 150 in the treated households) were randomly selected and interviewed.

The results showed that 25.3% (38/150) of householders in the control households were using insecticide treated bed nets. It was also revealed that higher percentage of householders in the control households (59.3%, 89/150) were using pyrethroid insecticides than in the treated households (31.3%, 47/150) and the difference between them was highly significant (Fisher's exact test: $p < 0.01$). The insecticides used included permethrin and deltamethrin which were bout from retailers in the village. The insecticides were mixed with water and then sprinkled on floors and walls to control domestic tick and other pest infestations. Other people mixed the insecticides with mud and then plastered on the entire house including walls and floors.

Other anti-tick activities mentioned by the respondents included plastering and sprinkling hot water of the house floors and walls. The use of these activities was not significant different between control and treated households (Fisher's exact test: $p > 0.05$). In total, 85% (127/150) of people in the control households were using anti-tick activities to control domestic tick infestations (25.3% used ITNs and 59.3% insecticides other than ITNs) (see table 4.3.10).

Table 4.3.10: Use of ITNs and other anti-tick activities in the control and treated households in April 2005 (26 months post intervention) in the study site

	Control households *(N = 150)*	Treated households *(N = 150)*	Fisher's exact test:		
			95% CI	*P-values*	*OR*[21]
Non use of anti-tick activities	23 (15.33%)	N/A	-	-	-
Use of ITNs	38 (25.33%)	149 (99.33%)	0.000 – 0.001	0.000	0.002
Irregular use of ITNs	N/A	29 (19.33%)	-	-	-
Non use of ITNs during dry/hot seasons and when no tick-bites)	5 (3.33%)	21 (14.00%)	0.060 – 0.601	0.002	0.210
Use of insecticides (other than ITNs)	89 (59.33%)	47 (31.33%)	1.940 – 5.290	0.000	3.200
Sprinkling hot water on floors and walls	24 (16.00%)	13 (8.67%)	0.930 – 4.480	0.078	2.010
Plastering of house walls and floors	57 (38.00%)	44 (29.33%)	0.890 – 2.460	0.142	1.480

4.3.11 Effects of net washing on ITNs efficacy

Frequency of net washes

The results showed that 53.6% (112/209) of householders did not wash their nets. The frequency of net washes ranged from 1 to 6 with a mean number of 3.5 ± 1.87 washes (SD) over three months post intervention began (See table 4.3.11a).

Table 4.3.11(a): Frequency of net washes in treated households in April 2004 (3 months post the trial began in January 2003) in the study site

No. of net washes Reported over three months	No. of respondents on net washes (N = 209)	% of respondents on net washes
0	112	53.59
1	39	18.66
2	21	10.05
3	18	8.61
4	11	5.26
5	6	2.87
6	2	0.96

[21] OR = Odd Ratio (total number of new cases/total number of population at risk)

Effects of washing on ITN efficacy

A field study was conducted in April 2003 in the study site to determine how often the ITNs were washed and the effects of washing on ITN efficacy against *O. moubata s.l.*

Mortality rates of *O. moubata s.l.* exposed to the positive control (fresh treated netting) ranged from 67.5% to 95% with the mean percentage mortality of 81.7 ± 13528 (SD). Mortality rates of *O. moubata s.l.* exposed to ITNs received two and three washes ranged from 35% to 80% with a mean percentage mortality of 53.3 ± 23.629 (SD) and 22.5% to 80% with a mean percentage mortality of 49.3 ± 28.746 (SD) respectively. The mortality rates of *O. moubata s.l.* exposed to ITNs received four and six number washes ranged from 28% to 65% at a mean percentage mortality of 45.3 ± 18.610 (SD) and 28% 25% to 40% at a mean percentage mortality of 31.0 ± 7.937 (SD) respectively (see table 4.3.11b). The results clearly showed that mortality rates of *O. moubata s.l.* exposed to treated netting decreased steadily with the increase number (frequency) of washes the nets received, thus washing of nets reduces the efficacy of ITNs against soft ticks (Figure 4.3.11).

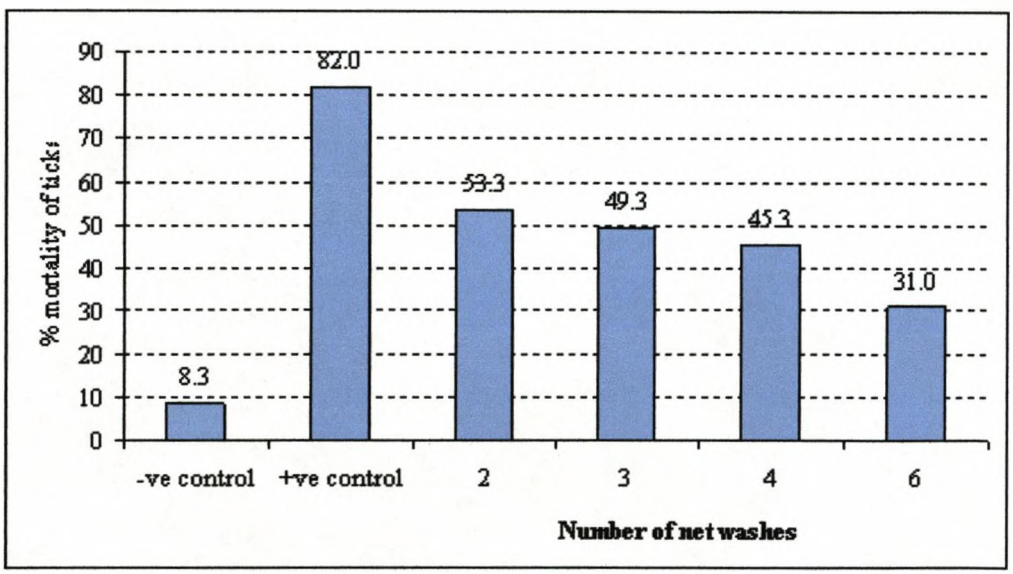


Figure 4.3.11: Mean percentage mortalities (mortality rates) of *Ornithodoros* ticks exposed to washed insecticide treated bednets compared to positive control

Table 4.3.11(b): Mortality rates of *Ornithodoros moubata s.l.* exposed to washed nets

Exposure time (min)	No. of ticks tested	MORTALITY RATES					
		Negative Control	Positive control	Number of net washes			
				2	3	4	6
15	40	2 (5%)	27 (67.5%)	14 (35%)	9 (22.5%)	11 (27.5%)	11 (27.5%)
30	40	2 (5%)	33 (82.5%)	18 (45%)	18 (45%)	17 (42.5%)	16 (40%)
60	40	6 (15%)	38 (95%)	32 (80%)	32 (80%)	26 (65%)	10 (25%)
Mean (%)		8.3	82.0	53.3	49.3	45.3	31.0
SD		5.774	13.528	23.629	28.746	18.610	7.937

4.4 DISCUSSION

This is the first study to evaluate the use of insecticide treated bed nets (ITNs) for vector control of Tick-Borne Relapsing Fever (TBRF). A randomised controlled trial of ITNs was conducted and, based on the substantial reduction of numbers of households infested, tick densities in infested households, prevention of tick-bites and incidence of *Borrelia* infections in children has shown that this method can successfully control populations of *Ornithodoros moubata s.l.* and prevent TBRF transmission.

The reduction in domestic tick infestations in the treated households was significantly greater than in the control households throughout the trial and the relative risk of domestic tick infestations in the treated households was six times less than in the control households. This demonstrates a strong consistent and sustained effect over the 29 months of the trial, which is very encouraging. The study has also demonstrated that even without raised beds, ITNs have a great effect on reducing tick feeding success and protecting people from tick-bites. Only 13.8% of ticks in the treated households were detected with fresh blood meals as compared with 44.8% in the control households. Also ITNs were reported by more than 88% of people in the treated households to provide protection from tick bites and domestic tick infestations (see section 4.3.9). Despite a small number of ticks still managed to obtain blood meals. Potentially, they may have fed on individuals outside the nets or from other animals, such as chickens or pigs (see section 2.3.6). Ticks also may have started feeding on human hosts at earlier hours before they went to bed.

The data are all the more convincing given the high numbers of people in the control group that were carrying out anti-tick activities. People from the control households likely learnt the importance of ITNs in protecting people from tick bites and domestic tick infestations from their counterparts in the treated households. High acceptance of the intervention provides further evidence that ITNs was useful and protective against

domestic tick infestations and tick-bites, and the high number of control households using ITNs encouraging (see section 4.3.9).

The study conducted in Dodoma Rural District in 1996 showed that indoor residual spraying with insecticide is an effective means of tick control as there was a total eradication of domestic tick infestations with an 86% reduction of TBRF cases in the treated village (Talbert *et al.*, 1998). However, indoor residual spraying (IRS) has a number of drawbacks as discussed by Curtis (1990; Guyatt *et al.,* 2002a) regarding the control of malaria, including the excito-repellent effect of insecticide, difficulty of access by spray teams to houses and unsightliness and smell of spray deposits. This method is also very expensive to sustain as it involves paying trained spray-teams which must be transported to cover all TBRF endemic areas in the country. In these circumstances, ITNs have important advantages, as well as being less demanding than IRS in terms of infrastructure and organization; ITNs allow vector control resources to be targeted toward those most at risk in stable endemic settings, i.e. pregnant women and young children, hence best use can be made of limited resources. ITNs can give protection of longer duration than IRS since a net in good condition gives reduced but still significant protection to the user even after the insecticide has worn off. This advantage will be further strengthened by the emerging development of Long Lasting Insecticidal Net (LLIN) technology, which greatly extends the effective life of the insecticide.

Several trials on the use of ITNs to control malaria vectors in Africa and elsewhere have been conducted and have proved beyond doubt that ITNs are more effective in reducing vector density and consequently reducing both malaria morbidity and mortality in children under five years old and pregnant women (Lines *et al.,* 1987; Lindsay *et al.,* 1988; Curtis *et al.,* 1996; Magesa *et al.,* 1991, 2005). Therefore, in Tanzania, ITNs provide better tool than indoor residual spraying (IRS) as they are already widely used for malaria control and showing they can also kill ticks will further enhance promotion and acceptance for malaria control.

The reduction in transmission that detected is based almost entirely on the data from April 2003 (four months after the trial began) when prevalence of *Borrelia spp.* infections in the treated households was 0.3% (2/780) compared with 1.7% (17/1019) in the control households. No further *Borrelia* infections were detected in the treated households, but 0.2% (1/517) cases occurred in April 2004 in the control. TBRF incidence in the Mvumi region is known to rise and fall in consecutive years though the reason why is not known. For example, in 2000, TBRF incidences were 0.8% and 0.96% in April and August respectively, while in 2001 the incidences were 2.3% and 1.96% for April and August respectively (Mvumi hosp data 1997 – 2001: *unpublished report*). Climatic changes might also be the cause for the variation of *Borrelia* transmission in the region; however it remains to be elucidated. Alternatively, the reduction in *Borrelia spp.* infections in the control households might have been due to changes in human behaviour and increase in anti-tick measures (including using insecticide treated nets and other insecticide-based interventions such as permethrin and plastering) in the control households. Community-wide effects of ITNs might also be the cause of the reduction of both domestic tick infestations and *Borrelia* infections in the control households which benefited from the adjacent treated households.

There is increasing evidence that high coverage of ITNs within a community reduces malaria transmission even to those people without the nets because of community-wide effects (Binka *et al.,* 1998; Maxwell *et al.,* 2002; Curtis *et al.,* 2003). The use of ITNs has been shown in Kenya to reduce the population of vector mosquitoes in large areas with a substantial community-wide effect to protect people without nets located within 300 metres of compounds with ITNs (Gimnig *et al.,* 2003).

This community effect on nearby compounds without nets is approximately as strong as the effect observed within villages with ITNs. This implies that in areas with intense TBRF transmission with high ITN coverage, the primary effect of insecticide-treated nets is might be due area-wide effects on the domestic tick population and not, as commonly supposed, by simple imposition of a physical barrier protecting individuals

from biting. However this remains to be determined. To maximize their public health impact, high coverage with treated nets is essential.

The dramatic difference in *Borrelia* infections between control and treated households in April 2003 is probably due to people in the control households not yet perceiving the effectiveness of ITNs in protecting people from tick-bites and domestic infestations. Thus, by 14 months after intervention, more than 47% of householders in the control group were using insecticides to control domestic tick infestations.

Notably there was a significant reduction of the prevalence of malaria infections in the treated households compared to the control households, confirming what is already well known about the effect of ITNs on malaria transmission (Curtis *et al.,* 1996; Magesa *et al.,* 2005; Maxwell *et al.,* 2006).

The study also showed that acceptance and satisfaction with the ITNs was high. The majority of householders (88%) in the treated group reported that they had not seen domestic ticks or other pests, such as fleas and bedbugs, since using ITNs. Also there were no reported adverse events attributed to the interventions. People from treated households reported high reduction of both domestic tick infestations and tick-bites in their households. Interestingly, perception of domestic tick infestations and tick bites in the study site was gender and age specific. Women reported more tick problems including domestic tick infestations and tick-bites than men, perhaps as women are the caretakers (as in most traditional African cultures) and spend most of their time in the home primary with the family. Adults (45+ years) were more knowledgeable on domestic tick infestations than younger persons. The findings also suggested that family size was positively associated with *Ornithodoros moubata s.l.* proliferation, implying that people from large families are exposed to a higher risk of tick-bites than people from small families, although this was not followed up.

Prior to conducting the trial, baseline surveys were carried out to establish the level of domestic tick infestations and *Borrelia spp.* infections in the study site. The results

showed that the prevalence of domestic tick infestations was 71.5%, somewhat lower than the 88% previously reported in the region (Talbert *et al.,* 1998), though still very high. The majority of people (89%) in the area sleep on floors using either matting or animal hides and more than 90% of houses are of the traditional 'tembe' style (rectangular with a flat roof of sod or earth supported by poles and walls of mud plastered wicker or sun-died bricks). These findings support Walton's earlier (1964) reports from Meru in Kenya, that these types of houses were heavily infested with *O. moubata s.l.,* which typically remains underground within the relatively cool and humid hiding during the day and emerge at night for feeding.

The study also showed that the prevalence of *Borrelia spp.* infections in children under five years old was very high in this area. However, 2.8% and 5.3% of children had *Borrelia* infections were detected by microscopy and PCR, respectively. This demonstrates the significant increase in sensitivity that can be achieved by PCR in detecting TBRF when compared to microscopy, which has been previously shown to be unreliable (Dennis, 1998). Prevalence rates of 7.5% of *B. duttonii* infections in pregnant women and 3% in children under five years old were reported in Dodoma rural district central Tanzania (Mushi, 1996), further confirming TBRF as a major cause of health problems in the region. The identification of a new species of *Borrelia* in that study (Kisinza *et al,* 2003) is not a subject of this thesis and is thus not discussed here.

Susceptibility of *O. moubata s.l.* to pyrethroids was established in the laboratory before the ITNs were introduced in the study site. The study showed that *O. moubata s.l.* are highly susceptible to pyrethroid insecticides, which was previously shown to be the case (Varma, 1964; WHO, 1989; Vasil'eva, *et al.,* 1992) and is not surprising, given the absence of insecticide use in this area until the trial. Repeated net washing reduced the efficacy of ITNs against ticks. Treated bed nets that received more than 4 washes over a period of three months were relatively ineffective against ticks, as mortality rates of ticks exposed to these nets was less than 45%. Similar studies conducted in Colombia and Bolivia on malaria vectors showed that the effectiveness of insecticide-treated materials (ITMs) for malaria control was reduced by washing (Ordóñez Gonzalez *et al.,*

2001). Other reports (Lindsay *et al.,* 1991; Curtis *et al.,* 1996; Jawra *et al.,* 1998; Adam *et al.,* 2002; Ordonez-Gonzalez *et al.,* 2002) from studies conducted elsewhere showed that repeated washing of insecticide treated bed nets reduces insecticide doses to below the level required to effectively controlling malaria transmission. However, given that net washing rates are often low in the area as majority (54%) of people in the area did not wash their nets; only 10% washed their nets three times over a period of three months. This is unlikely to be a major factor in limiting ITN effectiveness, in comparison with other factors, such as correct usage and re-impregnation.

The results suggest that if ITNs are used properly, they can reduce domestic tick infestations and subsequently prevent TBRF transmission in the disease endemic areas. For nets to be maximally effective, coverage must be high, nets should be re-treated promptly (or long-lasting nets used) and individuals should properly use their nets each night. Re-treatment schemes would become unnecessary if there was a complete switch over to the use of long-lasting insecticidal nets. Although a long lasting net may initially be more expensive, a switch over can be more cost effective over a four (Maxwell *et al.,* 2006) or perhaps a seven-year period (Tami *et al.,* 2004), the expense of annual re-treatment is avoided. Net distribution also needs to be accompanied by clear health education messages and encouraging community participation in the control programmes. The size and shape of the nets have to be adapted to the size and shape of the houses concerned, and the preferences of the people.

The other major challenge in the use of ITNs is that many of the people at greatest risk for vector-borne diseases such as malaria and TBRF are extremely poor and cannot afford to buy nets. Each illness episode costs family substantial resources, and additional economic problems arise if the disease affects the men or women in charge of the household. As TBRF is a disease of poor, a compelling argument can be made in favour of public-sector distribution of treated nets with special emphasis on protecting pregnant women and young children. Given the evidence presented here, ITNs can exert a significant impact on TBRF transmission, with the added benefit of protecting people against malaria transmission.

4.4.1 Study limitations

The ITN trial on vector control of TBRF in Tanzania encountered some challenges, including changes of human behaviour in the control households. Interestingly, frequently people in the control households started using insecticides such as Permethrin, which were bought from the shops and small medical stores in the village, mixed with water and sprinkled on the house floors and walls. Other anti-tick activities including plastering and sprinkling hot water on house floors and walls were reported from both treated and control households.

Also movement of people in the village from control to treated households and vice versa contributed to the improper use of ITNs. ITNs were sometimes moved to other control houses (*e.g.* when the child visits or because a relative lived there) confounding both groups.

Approximately 14% of the households in the treated group did not use the insecticide treated nets during dry and hot seasons when there was also high domestic tick infestations. This phenomenon has also reported for the use of ITNs in the control of malaria in Burkina Faso where there was a problem of using ITNs during dry and hot seasons (Frey *et al.,* 2006). Unfortunately, high domestic tick infestations occur during hot dry season with high *Borrelia* transmission likely primarily during the dry seasons when there is high tick-biting intensities (Walton, 1957).

CHAPTER 5

5.1 GENERAL DISCUSSION

The importance of Tick-Borne Relapsing Fever has clearly been overlooked and it is a truly neglected disease. This is clear, not only from the literature but it is reinforced by many of the findings presented here. Despite the absence of national data, it is already possible to state with some certainty that TBRF is a disease of major public health importance, in central and possibly in many other parts of Tanzania. There has yet to be a systematic survey conducted to establish the burden of disease in the country. Once that has been achieved, a strategy for TBRF control and prevention might be developed. It is now hoped that the findings of this study may assist in those developments.

This is the first study to be conducted on the natural history and vector control of tick-borne relapsing fever using insecticide treated bed nets (ITNs). As part of this study, new insight into the natural history of vectors, *Ornithodoros moubata s.l.* was gained and the opportunity was taken to explore the local population's knowledge, attitudes, perceptions and beliefs regarding TBRF transmission and control and how well such an intervention might be received.

The study clearly showed that ITNs are effective tools for vector control of TBRF as they will reduce or, in some cases, eliminate domestic tick infestations, prevent people from tick-bites and potentially reduce TBRF transmission. The reduction of domestic tick infestations in treated households was significantly greater than in control households throughout the trial. The relative risk of domestic tick infestations occurring in the treated households was six times less than in the control households at the end of the trial. The study also showed that acceptance of ITNs in the study site was high and there were no reported adverse events attributed to the intervention

Insecticide treated bed nets have been reported to be effective for control of malaria vectors in several countries (WHO, 1998). The use of ITNs has been shown in Kenya to reduce the population of vector mosquitoes in large areas with a substantial community wide effect (Gimnig *et al.,* 2003). In experimental hut studies conducted in Tanzania, pyrethroid impregnated bed nets have been shown to kill mosquitoes, to prevent others from feeding on humans and to drive mosquitoes out of houses (Magesa *et al.,* 1991). Similarly, in Papua New Guinea, Charlwood and Graves (1987) reported a marked reduction in the proportion of mosquitoes with blood-meals from humans using ITNs. It is now possible to add *O. moubata s.l.* to the list of vectors (Kroeger *et al.,* 2002, 2006; Pedersen & Mukoko, 2002) against which ITNs are effective.

Even without raised beds, ITNs were very effective in reducing tick feeding success and effective in preventing people from tick-bites. Only 13.8% of ticks in the treated households were detected with blood meals. Difference in the percentages of ticks detected with blood meals between control (44.8 %) and treated households (13.8 %) was highly significant. This suggests that ITN prevents domestic ticks from feeding on people sleeping under treated nets, thus protects people from tick-bites and consequently from *Borrelia* infections. Detection of small proportion of ticks with blood meals in the treated households suggested that ticks might have fed on domestic chickens if they were unable to feed on humans. This study showed that ticks in houses in Mvumi readily fed on chickens. It was also reported in Kenya that *Ornithodoros* ticks readily feed on domestic chickens to the exclusion of humans (Walton, 1964). This, and the fact that ticks readily move between pigpens and houses gives causes for concern as the same vector, *Ornithodoros moubata s.l.* can transmit *Borrelia* pathogens to both human and domestic chickens as well as pigs. However, data from the trial suggest that ITNs may effectively deal with this aspect of the vector's behaviour.

Our findings showed that a reduction in transmission of *Borrelia* infections is also possible through the use of ITNs. The apparent reduction of *Borrelia* spp. infections in the control households (if not simply a reduction in transmission throughout the region at the time of the study) might have been caused by changes in behaviour as people in

the control households started using various anti-tick measures. While this has confounded to some degree of efficacy demonstrated by the trial, it does indicate that ticks constitute such a major source of nuisance that people will accept interventions to prevent biting. This will impact very positively on efforts to increase ITN usage in Africa (Curtis *et al.,* 2003; Magesa *et al.,* 2005)

However, despite this complication, the study clearly showed that ITNs were effective in reducing prevalence of *Borrelia* spp. infections in children under five years old. Experimental surveys have confirmed efficacy of ITNs in terms of reduction of incidence of malarial disease (Lengeler 1998). These positive results concerning efficacy and effectiveness led to the recommendation and promotion of ITNs for malaria control.

The study also showed that acceptance of ITNs in the treated households was high. Majority of householders 88% in the treated group reported that they had not seen domestic ticks neither other pests such as fleas and bedbugs as used to be seen before using ITNs.

The perceived efficacy of ITNs for the reduction or elimination of domestic tick infestations and tick bites was also assessed. Domestic tick infestations were reported by 48% of the respondents in the control households, but by 12.1% of treated households. People from treated households reported reduction or cessation of both domestic tick infestations and tick-bites in their households.

Our findings clearly showed that distribution of *O. moubata s.l.* within households is heterogeneous as higher proportion of ticks was found in bedrooms than other sites of the houses (seating areas, kitchen and poultry areas). This presumably ticks are sedentary and prefer remaining in close proximity to their primary hosts in this case humans. The distribution of ticks in the bedrooms gives advantage of using ITNs not only to protect people from tick-bites but also maximizing the insecticidal lethal effects to host-seeking ticks, and probably can result into reduction of domestic tick population.

It has been reported in mosquitoes that they are naturally attracted to humans sleeping under nets and when alight on the nets, the insecticide kills them (WHO, 1989). Similarly, Geigy (1968) and Parola (2001) reported that ticks are attracted by the emanations and warmth of the human bodies and attractants.

This study clearly showed that *O. moubata s.l.* readily feeds on domestic chickens and all tick stages were equally fed (all tick stages transmits *Borrelia* pathogens). Walton (1964) reported in Kenya that ticks could readily feed on domestic chickens to the almost exclusion of man. Phipps (1950) also reported that ticks were found congregated to chickens habitats in Tanzania and suggested that possibly because of the warmth. Motshegwa (2004) showed that 11.7% of domestic chicken in Mvumi central Tanzania were infected with *Borrelia* spp. This is an important observation as it has shown that *B. duttonii* could be transmitted between hosts by the same vector. This suggests that for the control of TBRF has to be accompanied by promoting health education to change people's husbandry practices by refraining from keeping chickens inside human habitations.

The study also showed that ticks can move and re-populate human habitations after eradication from inside the household. This suggests that households adjoining pigpens are at risk of tick infestations. Motshegwa (2004) reported that 16% of pigpens in the villages around Mvumi hospital were tick infested. This shows that treating households alone may not be enough and pigpens may require treatment too to achieve control.

O. moubata s.l. is a nocturnal feeder (Walton, 1962), but its cycle of activity has never been recorded. Our study showed that *O. moubata s.l.* host seeking begins at 21:00 hours and gradually increases, peaking at midnight (2400 hours) after which the biting rates drops gradually to almost zero at 0600 hours. The same nocturnal biting pattern has been reported in malaria vectors, where biting rates peak at midnight (Lines *et al.*, 1991; Maxwell *et al.*, 1998; Pates & Curtis, 2005). Thus, as used for malaria intervention, ITNs will also be useful in protecting people from tick-bites and consequently TBRF transmission. Charlwood and Graves (1987) reported that ITNs

caused a shift of mosquitoes feeding behaviour to earlier in the night. Curtis *et al.* (2001) reported that there was a reduction in the numbers of *Anopheles funestus* bites per man per night following introduction of deltamethrin impregnated nets. In this case, for the *Ornithodoros moubata* ticks to change their feeding behaviour after introduction of ITNs remains to be determined.

The results also showed that tick biting intensity is seasonal. The higher intensity was found during dry than wet seasons. The explanation for this seasonal variation is mostly likely to be temperature and humidity related of the traditional ('tembe') houses which allow water to penetrate and during wet seasons rain reaches floors where soil becomes wets, colder and compacted which presumably prevent ticks from emerging at night for feeding. In Meru, Kenya, Walton (1957) reported that ticks were not found in the wet and cool climate because the grounds of traditional houses (huts) were also wet. Tick-biting occurring in all months, transmission of *Borrelia* infections can occur throughout the year with high transmission likely primarily during the dry seasons as during this time both domestic tick infestations and tick densities are higher.

The results on community's knowledge, attitudes and practices regarding TBRF showed that more than 14% of people in the treated households did not use their nets during dry seasons because as it was too hot to sleep under nets. This irregularity of net use may result of less protective against *Borrelia* spp. transmission. The same situation was reported in Burkina Faso where there was low compliance of young children using ITNs to protect from malaria during dry hot seasons (Frey *et al.*, 2006). Unfortunately, high domestic tick infestations occur during hot dry season with high *Borrelia* transmission likely primarily during the dry seasons when there is high tick-biting intensities (Walton, 1957).

5.2 CONCLUSION

Insecticide treated bed nets (ITNs) have been shown to be effective in reducing and/or eliminating domestic tick infestations, preventing people from tick-bites and substantial reduction of prevalence of Borrelia species infections in children under five years old. If used to prevent transmission of nocturnally transmitted vector-borne diseases, ITNs should also prove effective in the control of tick-borne relapsing fever in Tanzania. This demonstration of the further breadth in activity of ITNs can only assist in furthering the cause of ITN promotion in Africa.

The main drawbacks in the large-scale use of ITNs are human behaviour (resistance to use), cost of nets, the need for regular re-impregnation and difficulties in achieving widespread availability, and distribution (coverage). Recently, prices have decreased and promotion, delivery and affordability have improved with social marketing programmes (Magesa *et al.,* 2005). Nets are now produced locally and are more adapted to human needs in terms of quality, size, shape, colour, opacity. It has been proposed that they should be provided free of charge (Curtis *et al.* 2003) for example during the EPI vaccination programme, or as a 'kit for pregnant women' (Guyatt *et al.* 2002).

A technical solution to the re-treatment issue was recently found with the development of 'long-lasting nets' (LLINs) (Guillet *et al.* 2001), wash-resistant nets such as Olyset® (permethrin incorporated in polyethylene fibre) or Permanet® (deltamethrin loaded on polyester), which can sustains efficacy even after several years of use in the field (N'Guessan *et al.,* 2001).

But the major challenge remains that the cost of ITNs is a major barrier to ownership and usage for a large proportion of Africans who are among the poorest of the poor and also the most highly affected by endemic infectious diseases including malaria and TBRF. This must be addressed if this is to succeed.

5.3 AREAS OF FUTURE RESEARCH

Clearly, a wide range of topics in this field require further investigation, including improved methods for detection and identification of *Borrelia spp.* in the vertebrate host(s) and the relationship between west and east African TBRF epidemiologies. Much of this extends beyond my skills/ interests and will require concerted effort by teams of interested scientists and stakeholders. However, studies that might follow directly from work presented in this thesis however include:

5.3.1 Establishing the burden (the geographical distribution and prevalence) of TBRF in Tanzania (and ultimately, elsewhere in Africa).

5.3.2 Further studies on the efficacy of insecticide treated bednets for control of TBRF should be carried out, in different contexts, with a view to scaling up at national level.

5.3.3 The structure of the *Ornithodoros moubata* complex should be investigated in order to understand vector distribution, host preferences, vector-*Borrelia* relationships; such knowledge could facilitate the production of maps showing areas where TBRF risk might be predicted.

5.3.4 The knowledge gained by such investigations (5.3.3) would then enable studies on a range of aspects of tick natural history affecting vector competence, including host location behaviour, habitat and climatic preferences and dispersal mechanism and ability.

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APPENDICES

Appendex 1.0: Publications in Support of this Thesis

Kisinza, WN; McCall, P J; Mitani, H; Talbert, A; and Fukunaga, M (2003). A newly identified Tick-Borne *Borrelia* species and Relapsing Fever in Tanzania. *THE LANCET, **362,** 1283 -1284*

Kisinza, WN; Talbert, A; Culter, S; Siza, J; Jessey, P; Raymond, V & McCall, PJ (2004). Epidemiology and Control of tick-borne relapsing fever in Africa: Past, present and future research perspectives. *Tanzania Health Research Bulletin, **6** (1): 5 – 10*

Research letters

A newly identified tick-borne *Borrelia* species and relapsing fever in Tanzania

William N Kisinza, P J McCall, Harumi Mitani, Alison Talbert, Masahito Fukunaga

Tick-borne relapsing fever caused by the spirochaete *Borrelia duttonii* is a common cause of serious illness in central Tanzania. Screening of *Ornithodoros* sp ticks from infested houses for the presence of *B duttonii* had detected a previously unidentified species of *Borrelia*. We investigated whether this species infected the human population in a central Tanzanian village, by use of blood slide examination and PCR. PCR was twice as sensitive in detection of infections, showing *Borrelia* sp in six (11%) of 54 children with fever, and in 13 (4%) of 307 otherwise healthy children. Genotyping *Borrelia* from 17 infections identified *Borrelia duttonii* and an unnamed species. Our findings show that the newly discovered species is a causal agent of tick-borne relapsing fever.

Lancet 2003; **362:** 1283–84

Tick-borne relapsing fever caused by the spirochaete *Borrelia duttonii* is common in central Tanzania, where it can be a substantial cause of serious illness, mainly in children and pregnant women.[1] Although tick-borne relapsing fever is known throughout the country, the extent of the disease in Tanzania, and indeed elsewhere in Africa, remains to be ascertained.

In endemic villages in Tanzania, rates of house infestations with the *Ornithodoros* sp tick vectors can be very high (up to 88%)[2] and *Borrelia* sp infection rates in these ticks may be more than 60%.[3] Previous screening of ticks from infested houses for the presence of *Borrelia duttonii*, by use of PCR, detected a previously unidentified species of *Borrelia*.[3] We investigated whether this species infected the human population.

The village of Muungano is located about 12 km north-east of Mvumi hospital, which is 40 km southeast of the state capital Dodoma, in Dodoma Rural District, central Tanzania. Tick-borne relapsing fever is endemic in this area,[1,2] where it is one of the main causes of paediatric illness. To investigate prevalence of infection, blood samples were taken from two groups of children randomly selected from the population in Muungano during household surveys in October and November, 2002. Fingerprick blood samples were taken from children younger than 5 years with fever (axillary temperature >37°C associated with vomiting, feeling cold, joint or back pain, or headache) and from a random sample of afebrile, asymptomatic children. Informed consent was obtained from the parent or guardian of the child being tested. The ethics committees of the Tanzanian National Institute for Medical Research (Dar es Salaam, Tanzania) and Liverpool School of Tropical Medicine (Liverpool, UK) granted ethical approval.

Thick blood smears were prepared and dried at the village before staining with Giemsa and microscopic examination at Mvumi hospital. *Borrelia* sp infections were treated with PPF (procaine penicillin fortified with iron, 120 mg intramuscular injection daily for 5 days) and followed up for 7 further days. Individuals with *Plasmodium* were informed and directed to the nearest dispensary (Mvumi Makulu) for treatment. Blood was also spotted and dried onto small pieces of filter paper (approximately 5 mm diameter; Whatman 3MM, Maidstone, UK), put in labelled Eppendorf tubes with pierced lids, and stored in sealed plastic bags containing silica gel.

PCR based on a flagellin gene was used for detection of *Borrelia* sp; this highly conserved gene has been shown to be suitable for both detection and classification of the genus.[3] Positive samples were directly sequenced in both directions with an ABI3100 automated sequencer (Perkin Elmer, Boston, USA).

Of the 54 children with fever, three (6%) and six (11%) were found to have *Borrelia* sp infections by bloodslide and PCR, respectively (table). One individual's sample was positive by microscopy but not by PCR. Although the rate of malaria was high, only one individual was found to have both *Borrelia* sp (by PCR) and *Plasmodium* sp. Of the 307 randomly sampled afebrile asymptomatic children, six (2%) were positive by microscopy for *Borrelia* sp. *Borrelia* sp was detected in 13 (4%) of these individuals by PCR, twice as many as with *Plasmodium* (by microscopy).

	Malaria*	Tick-borne relapsing fever		Type of *Borrelia* sp				
		Blood slide	PCR	*B duttonli* type Ly	*B duttonii* type 2(B)	Unknown type 3	Unknown type 5	Unidentified
Children with fever (n=54)	15 (28%)	3 (6%)	6 (11%)	4	1	1	0	0
Healthy children (n=307)	6 (2%)	7 (2%)	13 (4%)	6	0	4	1	2
Total (n=361)	21 (6%)	10 (3%)	19 (5%)	10	1	5	1	2

*Only *Plasmodium falciparum* was detected.

Numbers of *Borrelia* sp and malaria infections

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A newly identified tick-borne Borrelia species and relapsing fever in Tanzania

N[illegible], P[illegible]

Tick-borne relapsing fever caused by the spirochaete Borrelia duttoni is a common cause of [illegible] illness in central Tanzania. Screening of Ornithodoros ticks from [illegible] caves for this pre-existing or 6 duttoni bed detection previously unrecogni[illegible] species of Borrelia. We investigated whether this species infected the human population in a central Tanzanian village by use of blood slide examination and PCR. PCR was twice as sensitive in detection of infections, showing Borrelia sp in six (12%) of 54 children with fever, and in 13 (4%) of 307 otherwise healthy children. Genetically Borrelia from 17 infections identified Borrelia duttoni and an unnamed species. Our findings show that the newly discovered species is a causal agent of tick borne relapsing fever.

[illegible] 2002; 360: 113–84

Tick-borne relapsing fever caused by the [illegible] Borrelia duttoni occurs [illegible] central Tanzania where it can be a substantial [illegible] of illness, mainly in children and [illegible] women.[1] Although tick-borne relapsing fever has been thought to occur throughout the eastern half of the African continent, and indeed [illegible] more in Africa, surprisingly little is known about its occurrence in individual countries.

In endemic villages the disease is [illegible] by periodic [illegible] Ornithodoros ticks, which can be found in [illegible] (up to 88%) and [illegible] sp infection rates in ticks may be more than 6·5%.[2] Previous screening of ticks collected from the [illegible] caves for the presence of Borrelia, by use of [illegible] detection, a previously undetected species of Borrelia, [illegible] whether this species infected the human population.

The village of Mtanga, [illegible] about 12 km northeast of Mvumi Hospital, which [illegible] to the district capital [illegible] Dodoma, Kondoa District, central Tanzania [illegible] undertaking [illegible] this area. Malaria is one of the most [illegible] causes of paediatric illness.[3] Investigation of [illegible] blood samples [illegible] from two groups of children randomly selected from the population [illegible] Mtunguru during

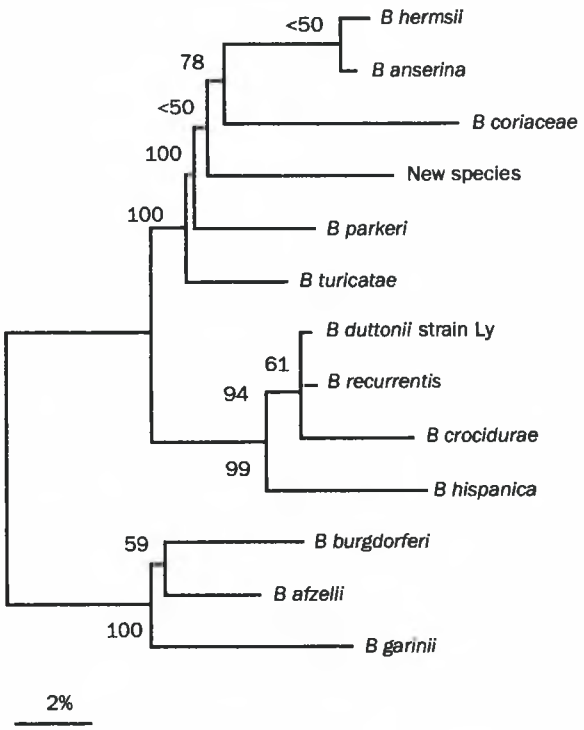
[illegible]

[illegible]	Tick-borne relapsing fever	
	Borrelia sp	PCR
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
Total	[illegible] (6%)	20 (6%)

*One Plasmodium falciparum was detected

Numbers of [illegible] sp and malaria infections

[illegible]	Type of Borrelia			
	Borrelia duttoni type 3	Unknown	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]



Phylogenetic tree of *Borrelia* species
Constructed from flagellin gene sequences with DNASTAR and CLUSTAL W software packages, with published sequence data.[3] Bootstrap values are shown and the bar represents a 2% difference between sequences. Sequences for the new species have been deposited in the DDBJ, GenBank, and EMBL databases (accession numbers AB105117–AB105133).

Two infections were detected by bloodslide but not by PCR, and one individual was found to have both *Borrelia* sp (by PCR) and *Plasmodium* sp. Thus, combined bloodslide and PCR screening detected *Borrelia* sp in 5% (15 of 307) of seemingly healthy children.

Genotyping was successful in 17 of 19 infections and a similarity matrix and neighbour-joining phylogenetic tree were constructed (figure). Of the four *Borrelia* types recorded in this investigation (table), two were identical to *B duttonii* strains Ly and type B.[3] The others, types 3 and 5, were identical to *Borrelia* type C isolated previously from *Ornithodoros* sp ticks in this area.[3] This is a new unnamed *Borrelia* species, differing from *B duttonii* and the other Afrotropical species, *Borrelia recurrentis* and *Borrelia crocidurae*. It is phylogenetically closer to the Nearctic *Borrelia* species (figure), particularly *Borrelia anserina*, to which it is most closely related (94·4% similarity in a 344 nucleotide sequence) and to *Borrelia parkeri*, *Borrelia turicatae* (93% similarity to both), and *Borrelia hermsii* (91·5% similarity). Of the 17 infections genotyped, 11 were *B duttonii* and six were the unidentified species. One child presenting with fever was infected with the unnamed *Borrelia* alone. In no cases were both *Borrelia* species found in the same host. All infections responded to treatment, without complications.

The discovery of this new *Borrelia* sp in human beings and in ticks collected within houses, in an area where tick-borne relapsing fever is endemic, suggests that the organisms causing such fevers in Tanzania are more complex than previously believed. Records of tick-borne relapsing fever in most villages within this study area[1,2] show how serious this infection is for the local population. Disease has always been attributed to *B duttonii*, and the relative importance and the clinical spectrum of each species must now be ascertained. Many further questions arise, including the possible existence of animal reservoirs, possible interactions, and differences in causes, epidemiology, and distribution between the species.

Our findings show that the sensitivity of screening for *Borrelia* sp infections can be increased by use of PCR. Since detection of tick-borne relapsing fever by microscopy is unreliable[4] and misdiagnosis is likely, the problem could be more common than records indicate. The rate of infection is likely to be even higher than that reported here, since during the hot months when the study was undertaken, the population slept outdoors, avoiding attack by the indoor-dwelling ticks. At Mvumi Clinic, monthly rates of *Borrelia* sp slide positivity can be over 7% (unpublished data), which our PCR data suggest is probably an underestimate. Moreover, the detection of *Borrelia* sp parasites in 4% of afebrile asymptomatic individuals (who, admittedly, might subsequently have developed fever) is very important and also needs further investigation.

With *B crocidurae* in west Africa,[5] there are clearly at least three *Borrelia* sp causing tick-borne relapsing fever in Africa, making the development of vaccines a difficult prospect. However, in East Africa at least, the fact that endophilic ticks transmit both species of pathogen indicates that vector control for prevention of this disease[2] is still a viable method.

Contributors
W N Kisinza did the fieldwork. P J McCall conceived and designed the study, and was principal investigator on the project. H Mitani and M Fukunaga undertook the PCR, DNA sequencing, and phylogenetic study. A Talbert supervised the fieldwork. P J McCall wrote the manuscript.

Conflict of interest statement
None declared.

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Epidemiology and control of tick-borne relapsing fever in Africa: Past, present and future research perspectives

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Abstract: Tick-borne relapsing fever is a neglected bacterial infectious disease caused by *Borrelia* species transmitted to human beings either by bites or coxal fluid contamination of *Ornithodoros moubata* complex ticks. The paper gives the insights of the epidemiology of tick-borne relapsing fever and its vectors in Africa, clinical characteristics of the disease, and problems of differential diagnostic techniques of the *Borrelia* parasites from both human and ticks. The challenges of a newly discovered pathogenic *Borrelia* species and the consequences of the inaccurate data on the distribution of TBRF in Tanzania are discussed. Treatment criteria and control strategies for the disease are presented. Effects of rapid changes in most environments of the world brought about by the population explosion and socio-economic factors on the natural endemicity of the disease intensity and distribution are discussed. Finally, priority research areas on TBRF and the way forward are highlighted.

Distribution of tick-borne relapsing fever

Tick-borne relapsing fever (TBRF) is a disease of man caused by bacterial spirochetes (*Borrelia* species), that are transmitted by soft ticks either through biting (saliva) or contamination of bite wounds with coxal fluid. Ticks acquire the spirochetes through feeding on an infected human host. Once the tick is infected, the spirochetes can be maintained for the lifetime of that tick, or to subsequent generations through transovarial transmission.

The disease has been known since ancient times and was recognized to be a tick-transmitted disease in 1905, when Dutton and Todd demonstrated spirochetes in *Ornithodoros moubata* ticks in West Africa (Dutton & Todd, 1905). The disease is present throughout Africa, with exception of the Sahara Desert and the rainforest belt. In West Africa, TBRF is caused by the spirochete *Borrelia crocidurae* and its geographical distribution is classically limited to the Saharan regions where the vector tick *Alectorobius sonrai* is distributed (Trape, 1996; Trape & Duplantier, 1991).

Trape & Duplantier (1991) showed that, TBRF is an endemic disease and a common cause of morbidity among older children (10 – 14 years) in rural villages in west-central Senegal with an average annual incidence of 1.6-4.2%, second to malaria infection. For children under five years old the relative importance of this disease is low, probably because of the high incidence of many other infections in this age group. It has also been shown that the high incidence of TBRF in human populations in West Africa probably reflects the close association of humans with the vector ticks and the rodent reservoirs.

Trape (1996) studied the spread of TBRF in West Africa and its relationship to sub-Saharan drought. He found that the annual incidence of the disease rose by a two-fold increase from that observed in 1991 (4.2%). The persistence of sub-Saharan drought is thus thought to be responsible for a wide spread of the disease in West Africa. Drought is incriminated in allowing the vector tick *A. sonrai* to colonize new Savannah areas. Thus global warming and its associated climatic changes are expected to affect a wide range of ecological processes, with consequences on vector-borne diseases transmission.

In Eastern and Southern Africa, TBRF is mainly distributed in the tropical areas, mainly Central Africa, Ethiopia, Madagascar, Kenya, Uganda, Tanzania, Somalia, Rwanda, Burundi, Democratic Republic of Congo, Malawi, Zambia, Zimbabwe and South Africa (Walton, 1964; Parola *et al.*, 1999). The main pathogenic agent of TBRF in these areas is *B. duttonii* whose natural vector is *O. moubata*, which lives in dirt and cracks of mud floors or in mud and grass walls of traditional houses. This tick was originally confined to the closed biotopes of warthogs and porcupines from where it was transferred, possibly by hunters, to human dwellings where it became well adapted and anthropophilic (Walton, 1962). The tick is sedentary and nocturnal feeder that remains within a radius of

30 m, although it may be transported greater distances on hosts.

East African TBRF has been the longest and most intensively investigated disease. It differs from the other tick-borne relapsing fevers because its vector, *O. moubata*, is a domestic ectoparasite, and man represents the only reservoir of the causative agent, *B. duttonii*. TBRF is a significant cause of infant and perinatal mortality and morbidity in East Africa. The mortality differs markedly between locations, ranging from 0–8% (Goubau, 1984), but pregnant women and young children appear to be more susceptible than other groups. For instance, in the Democratic Republic of Congo the incidence of TBRF among all pregnant women in the maternity ward was 64%, and this condition often led to maternal death or to spontaneous abortion. The prevalence of the disease in the outpatients ranged between 4.3% and 7.4%. In Rwanda, Goubau *et al.* (1983) showed that during pregnancy the TBRF is particularly intense and that pregnant women have high spirochete burdens, leading to high fever (Melkert & Stel, 1991) than non-pregnant women. Jongen *et al.* (1997) assessed the impact of TBRF on the outcome of pregnancy in Tanzania and showed that, the risk to births during the attacks of TBRF was 58.0%, with extremely high perinatal mortality (436 per 1000 births). The total loss of pregnancies including abortions was 475 per 1000, and the relapse rate was 3.6%, compared to 1.7% in non-pregnant women. The risk of delivery during the attack was positively correlated to increasing density of the spirochetaemia.

Recently, Jonathan (2001) has shown that, the prevalence of the disease is more common in males (60%) than in females. Barclay & Coulter (1990) also conducted a study on TBRF in Dodoma, Tanzania and found that, the annual incidence due to TBRF in one-year children was 38.4%, and in those under five years old was 16%. However, the prevalence of TBRF in children < 5 years in Dodoma was found to be 5 % (Kisinza *et al.*, 2003). Melkert & Stel (1991) showed that, TBRF is exceptionally rare in neonates, and the route of infection in infected neonates remains the subject of controversy. Trans-placental transmission is the most likely explanation for some of the affected neonates.

There are no accurate data of the number of cases of TBRF in Tanzania as it is rarely reported in the routine Health Management Information System of the Ministry of Health. Nevertheless, in 1996 the TBRF was reported from most regions (13/20) of the Tanzania mainland. This shows that, TBRF is widely spread throughout mainland Tanzania, but is likely to be grossly under-reported.

Although 65% of the regions of Tanzania are endemic for TBRF, actual magnitude of the disease in each district is hard to establish. In a recent review of the TBRF situation in Mwanza, Tanzania (2000-2002) data available (Table 1) showed that an overall total case fatality rate was 11.3%; with the rate for < 5years in Sengerema district alone being 24%. It is interesting to note that there were no mortalities in Ukerewe district during the 3 year period.

With the poor diagnostic service in most of the peripheral health facilities in Tanzania, it is likely that many cases of TBRF are classified as malaria, since it is difficult to clinically distinguish between these two diseases.

The very recent study on the epidemiology of tick-borne relapsing fever in Tanzania has disputed the notion that *B. duttonii* is the only pathogen of TBRF in East Africa, as a new pathogenic species of *Borrelia* has been isolated from children (Kisinza *et al.*, 2003). Phylogenetic analysis of this yet unnamed *Borrelia* species revealed that it differed from *B. duttonii* and the other Afro-tropical species (*B. recurrentis and B. crocidurae*). The new isolated species is phylogenetically closer to the Nearctic's *B. anserina*, *B. parkeri*, *B. turicatae*, and *B. hermsii* (Fukunanga *et al.*, 2002). The discovery of this new species in humans and in ticks collected within houses, in an area where TBRF is endemic, indicates that the organisms causing TBRF in Tanzania are more complex than previously believed.

Table 1: The incidence of TBRF in all age groups in Sengerema and Ukerewe districts of Mwanza region, Tanzania (2000 – 2002)

District	2000		2001		2002		Overall	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Sengerema	160	35	175	51	90	16	425	102
Ukerewe	249	0	179	0	50	0	478	0
Total	409	35	354	51	140	16	903	102
Case-fatality rate (%)		8.6		14.4		11.4		11.3

Vectors of TBRF in Africa

The main vector of TBRF is the *Ornithodoros* soft ticks, of which the species complex *O. moubata* is prevalent in sub-Saharan Africa, where it is found in many parts at < 2000 metres. It displays a marked preference for natural ground, especially for the powdered-fine loamy soil that is used in construction of huts (Geigy, 1968). *Ornithodoros* soft ticks stay quietly underground in the daytime and only emerge after sunset, and live in close proximity to humans in cracks and crevices of walls and floors of traditionally built houses.

Since Walton's (1964) work on the *O. moubata* complex in East Africa during the 1940-50s, few studies of any kind have been made on the vectors of TBRF in Africa. In particular, the taxonomy of this group has been ignored. Walton's classification of *O. moubata* into four major species, varying in their distribution and host preference, has been disputed but based on the comprehensive collection of material and observation of tick natural history from a wide geographical area it remains the most thorough investigation. Walton (1964) recognised *O. apertus* feeding on porcupines in Lake Naivasha, Kenya, *O. compactus* feeding on tortoises in South Africa, *O. moubata* and *O. porcinus* feeding on humans and warthogs occurring across a wide geographical range. Only the latter two were considered vectors of the disease. Both of these further comprised two subspecies or forms, i.e. a domestic form that lived in houses and fed on man and a wild form that typically lived in animal burrows and preferred animals other than man. However, the 'wild' form of *O. porcinus*, named *O. p. porcinus* may also live in human habitations and can readily feed on humans. Current molecular taxonomic methods could reveal the true structure of the species complex and facilitate the elucidation of the vectorial capacity of its member species. Superimposed on a thorough knowledge of the natural history of these sibling species, this would enable the distribution of the groups throughout Tanzania to be determined.

Few recent studies have reported the house-infestation ecology of *Ornithodoros* soft ticks in Tanzania. In a survey conducted by Talbert *et al.* (1998) in seven villages of Mvumi, Dodoma, 87% of the houses were infested with ticks. In a similar survey, Kisinza *et al.* (2003) conducted a study in 200 households in Muungano village, in the same district. They showed that, the house-infestation with *Ornithodoros* soft ticks ranged from 50% to 87%. The distribution of ticks in the houses was observed to be heterogeneous; bedrooms were heavily infested (76%), followed by sitting rooms (13%), poultry area (7%) and kitchen (4%).

Clinical characteristics of the disease

TBRF begins with an acute onset of high fever with chills, headache, myalgia, arthralgia and coughing (Soneshine, 1993). Haemorrhages, iritis or iridocyclitis, hepatomegaly or splenomegaly may also occur (Southern & Sanford, 1969). Other manifestations include abdominal pain, nausea, vomiting, diarrhoea and photophobia. A rash may occur at the end of the first febrile episode, and neurological findings are frequent and may be severe (Cadavid & Barbour, 1998). Jaundice occurs in 7% of patients, and the case-fatality rate is 2-5% (Southern & Sanford, 1969). In general, the primary episode lasts about 3 days and is followed by a second, shorter, milder episode 7 days later (Sonenshine, 1993). The resolution of the disease has been linked to antibody production against the different antigenic types presented by *Borrelia* in the successive relapses. Without treatment, the fever will spontaneously subside after approximately 3 days, followed by an afebrile period of around a week before a relapse of the fever occurs. Up to 13 febrile episodes may occur.

Diagnostic techniques

Diagnosis is established by the demonstration of *Borrelia* in peripheral blood of febrile patients. This test has a sensitivity of 70% when blood smears are examined by means of dark-field microscopy or when stained with Giemsa or Wright's stain (Parola & Raoult, 2001). Recently, a quantitative buffy coat analysis has been described as a very sensitive and specific technique for the detection of *Borrelia* in blood (van Dam *et al.* 1999). Serological assays are not readily available, and their diagnostic value is limited because of the antigenic variation shown by the *Borrelia*. Molecular methods have been described for the identification of the agents in blood samples (van Dam *et al.* 1999) and a flagellin gene-based Polymerase Chain Reaction (Nested-PCR) has been developed and identified to be most useful technique both for detection and identification of *Borrelia* parasites extracted from human being and soft ticks (Fukunanga *et al.*, 2002). The Nested-PCR technique has been found to be twice as sensitive compared to the blood slide techniques (Kisinza *et al.*, 2003) and this technique might resolve the high rates of misdiagnoses of TBRF cases. However, the technique

is yet to be available for routine purposes. Microscopy technique will therefore, remain the most cost-effective method for some foreseeable future.

Treatment and control

The commonest antimicrobial treatment includes use of Procaine Penicillin (PPF) injections, intramuscularly (IM) daily for 5 days (dose 30,000 MU/kg) or in adults, tetracycline given orally 500mg q.i.d for 5 days. In pregnant women, PPF at 0.8MU IM daily for 5 days or erythromycin 500mg q.i.d for 5 days are used. Jarisch-Herxheimer reactions may occur upon treatment and typically this may start 1 hour after commencement of treatment with a chill phase of 30 minutes, rigors, and rise in temperature, pulse and respiration rates and, blood pressure. The peak temperature occurs 2-3 hours after treatment and coincides with disappearance of spirochetes from the blood. The flush phase follows with intense sweating and falls in blood pressure. There is also a risk of cardiovascular collapse and death.

Control measures against the soft tick vectors of TBRF can be divided into physical and chemical methods. Physical methods include short term measures such as sprinkling of hot water onto floors and walls to kill the ticks, and mixing water and earth-soil to plaster the surfaces of walls and floor to render them smooth surface and eliminate the cracks and soft earth where the ticks prefer to hide. The long-term solution would be to make floors and walls solid with cement mixtures instead of traditional wood and earth-soil construction. However, many families are unable to afford the costs of cement and other building materials.

Chemical measures involve use of acaricides of the synthetic pyrethroid group. Locally used insecticides that have been found to be active against ticks are lambda-cyhalothrin, permethrin and deltamethrin (Talbert *et al.*, 1998). Insecticide treated mosquito nets have also been found to protect against tick bites (Talbert *et al.*, 1998). Some householders have used the deltamethrin, supplied in net treatment kits, diluted with water, to sprinkle on the floor and walls. Methods used in the control of TBRF in Tanzania are summarised in Table 2.

Preliminary research has shown that using pyrethroid insecticides either for indoor residual spray or on mosquito nets is effective in reducing TBRF cases. Furthermore, improving house standards and using a mixture of lime and sands has been also useful in Dodoma district (Table 2). The annual costs for a household are TShs. 4,400 and TShs. 5,000 (US$4.4 and 5.0) for residual spray and treated nets, respectively. Although the cost for plastering of the floor and wall with limestone is less that TShs. 2,000,

Table 2: Traditional methods used to control TBRF transmission in Tanzania

Method	Cost	Effectiveness	Simplicity
Traditional plastering	+	+	+++
Insecticide-treated nets	+++	++	+++
Indoor residual spraying	+++	++++	++
Lime/cement flooring	++++	+++++	+

its application is very difficult and not cost-effective method. To prevent TBRF, insecticide treated mosquito nets would be the most cost efficient method. Currently in Tanzania, bed-nets could be provided at a cost of $3-5, with re-treatment costing $0.50. The cost of residual spraying was estimated in 1998 at US$7 per cycle of treatment (Talbert *et al.*, 1998), with re-treatment required every 1-2 years. The cost of laying a new floor would cost $30.

Conclusion

The magnitude of TBRF remains unknown in many African countries including Tanzania. In poor rural communities, TBRF remains undiagnosed, or is often misdiagnosed as malaria because it is difficult to clinicall distinguish between the two diseases. Misdiagnoses can contribute to the overuse of antimalarials, failure to treat TBRF and undoubtedly leads to the under-estimation of the problem. To overcome this problem, reliable simple standard case definitions for both community and facility levels are immediately needed.

TBRF persists in Tanzania where people live in traditional muddy dwellings. The low standards of traditional housing, aggravated by outmoded traditional designs and methods of construction, lie .

at the very root of tick infestation problems (Walton, 1962). Very often lack of water supplies during dry season compromises control methods as they rely on water.

The high perinatal mortality rate and morbidity in children under 5 years old and pregnant women due to TBRF calls for prevention and early effective management of the disease. This is the greatest challenge where access to health services in rural areas of developing countries is hampered by many factors. Also there is a great challenge of initiating sustainable community based health care programmes in rural areas where TBRF is endemic.

Areas to be addressed by future research should focus on the epidemiology of the disease risk factor analysis; vector competence for the spirochetes; the reservoirs of infection; host-microbial interactions; sub-clinical infection pathogenic mechanisms, effect of insecticide treated nets and tick identification. Other areas include vector bionomics, climatic conditions and social behaviour of the community at risk. Once we have data on these aspects, a mathematical model could be established through which intervention strategies could be explored prior to testing in the field. Evaluation of the success of these methods will only be possible through comparison with sound baseline disease information.

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Appendix 1.1: Presentations (Abstracts) in support of this Thesis

Kisinza, WN, Talbert, A & McCall, PJ (2003). Prospects of using Insecticide Treated Nets for Vector Control of Tick-Borne Relapsing Fever in Tanzania: Results from Baseline Surveys: *A paper presented at the Second International Conference on Tick-Borne Relapsing Fever held in Dodoma, Tanzania (12th – 13th August 2003).*

Kisinza, WN & McCall, PJ (2004). Epidemiology of Tick-Borne Relapsing Fever in Tanzania: 'A dogma of neglected diseases' *Proceedings of the 19th NIMR Annual Joint Scientific Conference held at AICC in Arusha Tanzania (15th – 17th March 2004).*

Prospects of using Insecticide Treated Nets for Vector Control of Tick-Borne Relapsing Fever in Tanzania: Results from Baseline Surveys*

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ABSTRACT

Tick-borne relapsing fever (TBRF) is a disease of man caused by spirochetes (*Borrelia spp),* transmitted to man by *Ornithodoros* soft ticks either through biting (saliva) or contamination of bite coxal fluid. Ticks acquire the spirochaetes through feeding on an infected host. Once the tick is infected, the spirochete can be maintained for the lifetime of that tick, or to subsequent generations through transovarial transmission. Although TBRF occurs throughout the country, the extent of the disease in Tanzania, and indeed elsewhere in Africa, remains to be determined.

Baseline survey was conducted in Muungano village between October and November 2002 to establish the level of domestic infestations and prevalence of *Borrelia* infections in the area. Both thick blood smear and PCR methods were used to screen a total of 361 children under five years old. In order to establish level of tick infestations, 200 households were randomly selected in the village and standard method of recovering ticks from infested households was used to collect ticks from four sites within each selected house (bedrooms, seating areas, and kitchen and poultry areas).

The results showed that of 54 under five children with fever, 5.6% and 11.1% were found to be infected with *Borrelia* by blood slide and PCR respectively. Prevalence of domestic tick infestations ranged from 50% to 87% and the distribution of soft ticks in house was heterogeneous. Bedrooms were heavily infested (76%) followed by seating areas (13%), poultry area (7%) and kitchen (4%).

Based on the high prevalence of *Borrelia* infections and domestic tick infestations, TBRF is likely to be one of the major public health problems in the area. And since ticks are nocturnal feeders found aggregated in the bedrooms, ITNs are likely to be important tool for vector control of TBRF with an added advantage for malaria control in Tanzania.

* A paper presented at the 2nd International Conference on Tick-Borne Relapsing Fever held in Dodoma, Tanzania (12th – 13th August 2003).

The epidemiology of Tick-Borne Relapsing Fever in Tanzania*: 'A dogma of neglected disease'

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ABSTRACT

Tick-borne relapsing fever (TBRF) is a 'neglected' bacterial infectious disease caused by *Borrelia* pathogens transmitted to humans either through bites or coxal fluid contamination by soft ticks. Soft ticks are the disease vectors belonging to the genus *Ornithodoros,* of which the species complex *Ornithodoros moubata* is prevalent in sub-Saharan Africa. TBRF is known throughout the country, but disease epidemiology and ecology of *Ornithodoros moubata* complex remain to be ascertained. Each *Ornithodoros* soft tick species has preferred environmental conditions and biotopes that limit the geographical distribution and, consequently, the risk areas for TBRF.

Pregnant women and children are most susceptible to the disease; incidences have been recorded at up to 38% in 1 year olds and 16% in 1-5 years olds; *B. duttonii* prevalence rates of 7.5% in pregnant women and 5% in under 5 year olds have been recorded in Tanzania; risk of pregnancy interruption can be as high as 30%.

High infant and perinatal mortality and morbidity as a result of TBRF in Tanzania, calls for prevention and early effective management of the disease especially in rural areas of developing countries where access to health services is hampered by many factors. An urgent action is needed on designing and putting in place some community-based health care interventions in areas where the disease is endemic.

Besides the typical similarities in clinical symptoms between TBRF and malaria, in most cases epidemiology of these diseases overlaps. As a result of this, TBRF is commonly misdiagnosed and treated as malaria. This contributes to the overuse of antimalarials, wrong treatment to TBRF cases and undoubtedly leads to the underestimation of the disease-burden. A recent discovery of a new pathogenic tick-borne *Borrelia* species in Tanzania is also a great challenge to the scientific community as to whether TBRF should continue being dogmatized as a 'neglected' disease. With *B. crocidurae* in West Africa, and *B. duttonii* in Central and East Africa, there are clearly at least three *Borrelia* species complexes causing TBRF in Africa, making the development of vaccines a difficult prospect.

* **Proceedings of the 19th NIMR Annual Joint Scientific Conference held at AICC in Arusha Tanzania (15th – 17th March 2004)**

Appendix 2.3.3: Mean number of ticks of different development stages per infested household

Tick stages	Total No. of ticks in the infested households	Mean No. of ticks per infested households (N = 143)	SD
Stage-1	255	1.78	0.440
Stage-2	260	1.82	0.450
Stage-3	313	2.19	0.542
Stage-4	165	1.15	0.284
Total	993	6.94	1.716

Appendix 3.3.3: Main causes of illness perceived by householders in the study site

Illnesses	No. of responses	Percentage (N=198)
Malaria	170	85.86
Tick-borne relapsing fever	120	60.61
Pyrexia (fever of unknown origin)	40	20.20
Diarrhoea	14	7.10
HIV/AIDS	1	0.51
Others *(Coughing, eye infection, abdomen pains & pneumonia)*	9	4.55

Appendex 4.2.7: DATA SHEET FOR DOMESTIC TICK SURVEYS

Form No. _____ Date of survey _____ Name of data collector _____

Hamlet ID No.	Household ID No.	Total No. of ticks sampled	Bedrooms				Seating areas				Kitchen				Poultry areas			
			Stage-1	Stage-2	Stage-3	Stage-4	Stage-1	Stage-2	Stage-3	Stage-4	Stage-1	Stage-2	Stage-3	Stage-4	Stage-1	Stage-2	Stage-3	Stage-4

Appendex 4.2.9: **DATA SHEET FOR SCREENING OF BLOOD SAMPLES**

Form No. _____ Date _____ Name of recorder _____

Study Group: (1) Treated households [ ] (2) Control households [ ]

S/N	Hamlet	Name (Children)	ID No	Sex (F/M)	Age (Years)	Additional information enquired from the parent/guardians			Results		
						Use of ITNs	Use of Antibiotics	History of Travel	B/S		PCR
									Malaria	*Borrelia*	*Borrelia*
1											
2											
3											
4											
5											
6											
7											
8											
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Appendix: 4.2.12: Structured interview questionnaire

HOUSEHOLD INTERVIEW QUESTIONNAIRE ON CONFOUNDING FACTORS FOR THE ITN TRIAL ON VECTOR CONTROL OF TBRF TRANSMISSION IN TANZANIA

(a) General information

Date: _____/____/_____
Hamlet: .....
Name of Respondent .....
Sex of the Respondent: (1) Male (2) Female { }
Age of the Respondent _____ (years)
Level of education: (1) primary school, (2) Secondary school, (3) University/College, (4) Non { }
Main Occupation of the respondent (1) Farming, (2) Formal Civil/Private employee, (3) Retail Business (4) others (specify)..... { }
Number of people currently living in the Household _____

(b) Use of bed nets

1. Does this household have bed nets? (1) Yes, (2) No { }
2. If YES, what type of bed nets? (1) Insecticide treated nets; (2) un-treated nets
3. Does your family regularly use nets? (1) Yes; (2) No { }
4. If NO (Q3), Explain .....
5. Who sleeps under the bed nets? (1) Father only; (2) Father & Mother, (3) Children only (4) All family { }
6. If some members of the households do not sleep under nets, what are the reasons? .....
7. Are there specific times or seasons when bed nets are not used? (1) Yes, (2) No, (3) Do not know { }
8. If YES, what are the reasons of not using?
   a. When there are more visitors in the households (too many people in the house) { }
   b. During hot seasons either seasonally (too hot) { }
   c. No insects' biting { }
   d. Other reasons (mention them) { }

(c) Use of anti-tick activities other than bed nets

9. Do you use any insecticide in this household? (1) Yes, (2) No { }
10. If YES,
    a. Mention the type of insecticide used (name) .....
    b. How is used and for what purpose (explain).....
    c. How frequently does the insecticides used? (_____days per month)
11. How long have you been using insecticides in your household? _____ (months)
12. How did you learn about the use of insecticides for the above-mentioned purpose?
13. Where did you get the information on the use of bed nets/insecticides to control malaria/TBRF? .....
