# Determination of tick-pathogen interactions during acquisition and transmission of *Borrelia duttonii* by *Ornithodoros moubata*

Bachelor's Thesis

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České Budějovice 2020

Cetkovic, A., 2020: Determination of tick-pathogen interactions during acquisition and transmission of *Borrelia duttonii* by *Ornithodoros moubata*. Bc. Thesis, in English – 45 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

**Annotation:** The goal of this thesis was to investigate the interaction between *Borrelia duttonii*, a causative agent of TBRF in Africa, and its tick vector *Ornithodoros moubata* in terms of acquisition and transmission dynamics. The infectivity of frozen sera and infected unfed *O. moubata* ticks was evaluated. The acquisition efficiency of *B. duttonii* 1120K3 and Ly isolates by *O. moubata* was determined and compared. The minimum acquisition time was established as well. Moreover, the sensitivity of Borrelia in the presence of different animal sera was analysed.

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# Abstract

The aim of this work was to investigate the acquisition and transmission dynamics of *Borrelia duttonii*, a common cause of relapsing fever in Africa, by the vector *Ornithodoros moubata*.

We assessed the infectivity of frozen mouse sera and determined that no infection was established in a naïve host despite the fact that the survival of *B. duttonii* at -20°C over period of several months was confirmed.

By needle inoculation of naïve mice with the infected, unfed tick-homogenate, we demonstrated that the expression of *Vmp* gene is continuously upregulated. We concluded that the Borrelia in unfed ticks are still infectious for a mammalian host over the course of several months. In addition, we attempted to quantify the level of *Vtp* expression by real-time, quantitative PCR.

The efficiency of acquisition of *B. duttonii* Ly isolate by *O. moubata* was determined to be considerably low. Its transmission dynamics by *O. moubata* was investigated as well.

The infectivity and acquisition of *B. duttonii* 1120K3 and Ly isolates were compared and the observed differences were identified.

Furthermore, we evaluated the acquisition ability of *B. duttonii* 1120K3 by *O. moubata* by allowing ticks to feed on infected mice. We determined the acquisition time threshold of 2.5 minutes.

The sensitivity of Borrelia to various animal sera was assessed. We concluded that the rabbit serum has a substantial advantage in growth efficiency of *B. duttonii* compared to pig and chicken.

# Acknowledgment

I want to give a special thank to my supervisor Dr. Ryan Rego for his support and guidance throughout my work and to him and prof. RNDr. Libor Grubhoffer, CSc., Hon. D.Sc. as well for the opportunity to work in the Laboratory of Molecular Ecology of Vectors and Pathogens.
I want to acknowledge the other members of the laboratory Helena Rohackova and Martin Strnad for their help and support and especially Zuzana Vavrušková who provided enormous help with the work done in animal facility.
I want to thank my laboratory colleagues Lisa Hain, Maximilian Bayer and Peter Weber for their help and motivation and without whom my work in the lab would not be possible. Lastly, I want to give a special thank to my whole family who provided me with the opportunity to study and who supported me through all these years of studying. My studies would not be successfully completed without them.

# 1. Introduction

## 1.1. *Borrelia* genus

*Borrelia* genus is contained within the family *Borreliaceae* belonging to the Spirochaetes phylum. *Borrelia* genus comprises *Borrelia* species associated with Relapsing Fever disease and another group of species related to Lyme disease or Lyme borreliosis. This genus has unique genetic characteristics compared to other prokaryotic organisms [1,2].

*Borrelia* spirochetes have distinctive cell morphology which cells are helical in shape, consisting of a protoplasmic cylinder enclosed by an outer membrane. A cytoplasm surrounded by the inner cell membrane and the peptidoglycan layer constitutes a protoplasmic cylinder complex. *Borrelia* are motile bacteria with the flagella placed in periplasmic space [2].

## 1.2. Relapsing Fever *Borrelia*

Relapsing Fever (RF) is a vector-borne disease caused by Relapsing Fever *Borrelia* species. Determined by the type of a vector, two forms of RF are recognized: tick-borne relapsing fever (TBRF) and louse-borne relapsing fever (LBRF) [3, 4]. TBRF is transmitted by argasid vectors or 'soft ticks' and rarely by ixodid vectors or 'hard ticks'. More precisely, there is currently only one RF Borrelia namely *B. miyamotoi* which is vectored by hard ticks of the Ixodes genus [5–7]. In addition, the human body louse is identified as a vector of LBRF. Tick-borne RF is a widespread disease identified in some parts of Europe, Africa, Central and Western Asia as well as in South and North America but is still being understudied and underestimated [4,5,8–10]. Most reported cases of TBRF in Europe/Asia are caused by the infection with *B. hispanica*, commonly described in southern Spain, and *B. persica* are endemic to southwestern Europe and the Middle East. However, many other RF *Borrelia* species such as *B. caucasica*, *B. turkmenica* and *B. baltazardii* have been identified in these regions as well. More precisely, *Ornithodoros verrucosus*, the vector transmitting *B. caucasica* has been found in two regions of Ukraine [11,12].

The classification of RF *Borrelia* species is performed based on the specificity of their interaction with a vector associated with a particular geographical area. Most current studies involve the research on the New World species *Borrelia hermsii*, *B. parkeri* and *B. turicatae* which are endemic in the regions of North America and are transmitted by *Ornithodoros*

*hermsii*, *O. parkeri* and *O. turicata* respectively. In Africa, most prevalent *Borrelia* species are *B. duttoni* and *B. crocidurae* classified as Old World RF *Borrelia* [4,6,9,10,13–15].
The size of RF *Borrelia* genome is about 1-1.5 Mb. It includes a 160 kb linear megaplasmid which possesses conserved regions which are not present in Lyme disease *Borrelia* species. In addition, some genes found in megaplasmid of *B. turricatae* are coding for some lipoproteins which are found to be associated with its interaction with a vector and a host [13].

### 1.2.1. Old World RF *Borrelia*

Old World (Afrotropic-Palearctic Ecozones) RF *Borrelia* and New World (Nearctic Ecozone) RF *Borrelia* are categorized as two separate clades based on their phylogeny and the regions of endemicity. In Africa, most prevalent *Borrelia* species are Old World RF *Borrelia* [16, 17]. First described representative of this group is *B. recurrentis* transmitted by *Pediculus humanus* and it is the only RF *Borrelia* species which vector is identified as a human body louse [4,9,13,18]. It is a cause of epidemic relapsing fever in Ethiopia and Sudan, even though the cases of LBRF were reported worldwide [9,13].
Additionally, another member of Old World RF *Borrelia* is *B. duttonii* detected in *O. moubata* ticks found in East, Southern and Central Africa, predominantly in Tanzania and Democratic Republic of Congo [13,14]. These African RF *Borrelia* species, together with *B. crocidurae* having *O. sonrai* tick as a vector, have the average nucleotide identity of 99% suggesting that they are closely related species [17]. Consequently, based on phylogenetic analysis and sequencing of complete genomes, it was concluded that *B. recurrentis* is a subset of *B. duttonii* and it was characterized as its louse borne adaptation [4,13,17].
Moreover, *B. persica* transmitted by *O. tholozani* was identified in Middle East, India and Central Asia [13]. In the regions of Morocco, Algeria and Tunisia, endemic TBRF is caused by *B. hispanica* [4]. In Europe, more precisely in Spain, Portugal and Greece, the prevalence of *B. hispanica* and its associated arthropod vector *O. erraticus* tick was recognized as well [13,19]. However, *B. persica* as well as *B. crocidurae* have also been identified as one of the main causes of TBRF in Europe [13]. Even though the infection with *B. hispanica* is sometimes accompanied with neurological problems, the mortality rate with *B. duttonii* is much higher and besides neurological, ocular complications and the newborn's infection can occur [4,13].

## 1.3. Relapsing fever

### 1.3.1. Course of disease and clinical characteristics

Upon an infection initiated by a tick-bite, the number of *Borrelia* increases, causing a first febrile episode. This usually occurs within the range of 4 to 18 days of incubation [5,9]. It corresponds to a first spirochetemic peak and is characterized by a high fever reaching the temperature of 41°C in some cases [13,20]. Compared to Lyme disease spirochetes, RF *Borrelia* can reach density of $10^7$/mL of blood during the highest point of spirochetemia [2]. After raising antibodies to fight spirochetemia, a host experiences the end of the first febrile episode, known as afebrile period. Since *Borrelia* are capable of escaping the host's immune response, another febrile episode occurs. This interchangeable pattern between febrile and afebrile periods results in multiple febrile relapses. The return of second spirochetemia is regarded to the ability of *Borrelia* to change the expression of their outer membrane proteins and thus lead to occurrence of a new serotype. This phenomenon, known as antigenic variation, is demonstrated in RF *Borrelia* species [15,20–23].

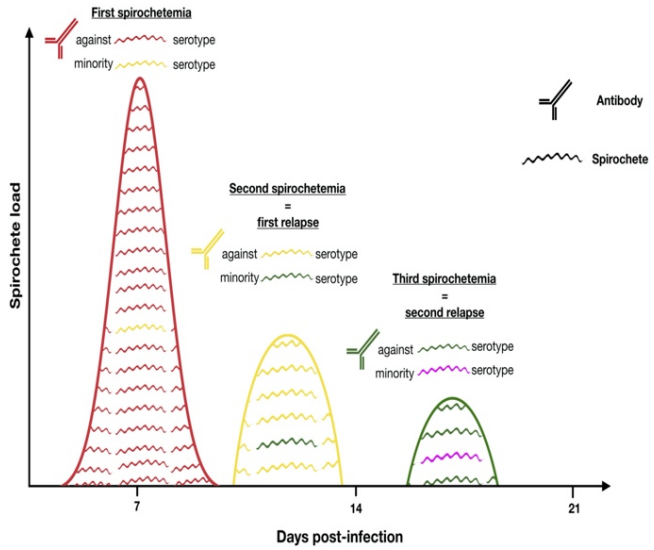


Figure 1. Course of infection within the host, adapted from [13]

Apart from the episodes of high fever, the other symptoms caused by TBRF are commonly flu-like including headache, tachycardia, arthralgia, nausea and possible skin rashes [13, 15]. In Tanzania, the mortality rate in humans caused by an infection with *B. duttonii* was determined to be between 2 and 3%. However, the antibiotics like penicillin and tetracycline used in treatments often have as a consequence notable side effects like Jarisch-Herxheimer reaction which causes that the mortality rate of 5% [15]. Additionally, the infant mortality rate

of up to 38% as well as the high risks of perinatal deaths have been observed in endemic regions, more precisely in Tanzania and Democratic Republic of Congo [14].

### 1.3.2. Diagnosis

TBRF is confirmed by the detection or isolation of *Borrelia* from a patient's blood in the laboratory [24]. Moreover, a microscopical detection is performed by placing a drop or the smear of blood on a glass slide and using a bright-field microscope to examine sample stained with a Giemsa stain. [9].

Cultivation of spirochetes in vitro is accomplished in a medium provided by Kelly [2,25]. Re-isolation of living spirochetes from a blood is achieved by putting a few drops of blood sample in a medium and incubated at the temperature between 30°C and 37°C. Cultures are usually checked under a dark-field microscope [9]. The majority of Borrelia species can be detected by the amplification of a DNA segment in polymerase chain reaction as well as the markers in genomic DNA which are specific for each species [26]. The infection of ticks can be confirmed by isolation in media or using naïve laboratory mice. In addition, to investigate transmission and acquisition ability, ticks can be allowed to feed on mice [9].

## 1.4. Antigenic variation in RF *Borrelia*

Antigenic variation of several RF *Borrelia* species represents an efficient mechanism for evading host's immune response, thus causing a persistent infection in the blood. While residing in the blood stream of a mammalian host, the *Borrelia*'s *Vmp* gene is switched on, indicating the expression of Variable major proteins (Vmps). However, the production of Variable tick protein (Vtp) is terminated in the blood. On the contrary, following the acquisition by ticks, these two protein expressions are reversed. Therefore, the expression of *Vtp* gene is upregulated while the *Vmp* expression is downregulated. This switching is controlled by environmental factors such as the concentration of spirochetes acquired by ticks, temperature and pH [20,22,23,27–29].

Additionally, the *group* Vmps further divides into two claases of proteins classified as variable large proteins (Vlps) and variable small proteins (Vsps). The size of large Vmp proteins is in the range of 37-40 kDa, while that of small is around 20kDa [30,31].

Additional strategy employed by RF *Borrelia* species to avoid specific immune response is

erythrocyte resetting, the binding interaction of spirochetes and erythrocytes through which spirochetes are covered with red blood cells and therefore undetectable by B cells [32,33].

## 1.5. Soft ticks (*Argasidae*)

The ticks of family *Argasidae* ("soft ticks") within the genus *Ornithodoros* are the vectors of most TBRF *Borrelia* species [14]. Together with the lineage *Ixodidae* ("hard ticks"), they belong to the order Parasitiformes and are defined as obligate parasites. *Argasidae* developed substantially different biological adaptations in comparison to the family of hard ticks as a result of distinct lifestyle [34–36].

The morphology of soft ticks differs from that of hard ticks by lacking a scutum and having leathery integument. The folds in an integument enable it to stretch while ticks are feeding on the host. This structural characteristic allows rapidly feeding argasid ticks to engorge in several minutes up to an hour or two [37].

Argasid ticks have unique life cycle, with generally 2-8 nymphal stages before moulting into an adult, contrary to only one nymphal stage in ixodid ticks. The development is regulated by the blood meal size and a temperature. After embryogenesis, six-legged larvae are hatching from the eggs and experiencing a true metamorphosis which occurs after a bloodmeal, with the exception of some larvae of *Ornithodoros moubata* complex. Larval moulting results in 8-legged nymph which undergoes several nymphal instars before moulting into an adult. Each nymphal developmental stage is completed within few weeks [34–40].

The life cycle of *O. moubata* showing four nymphal stages is illustrated in Figure 2. The moulting into next nymphal stage is triggered only after a received bloodmeal. In comparison to hard ticks, the mating of soft ticks occurs off the host and the mated, adult females lay several egg batches (few hundred eggs) after each bloodmeal. They can feed and thus reproduce multiple times during their lifespan in contrast to adult ixodids which die after laying eggs. Additionally, females may oviposit without being previously copulated due to an ability to preserve sperms [34–39,41].

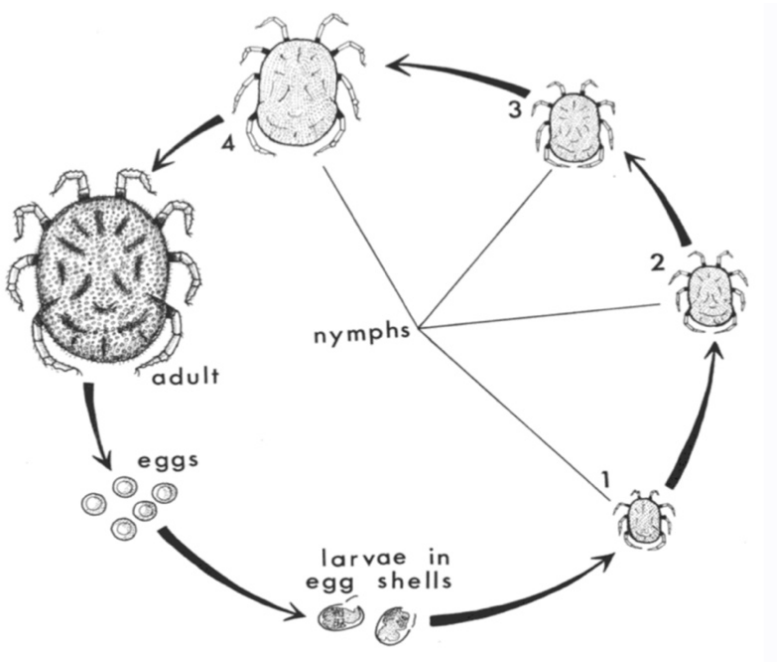


Figure 2. *Ornithodoros moubata* – four nymphal stage life cycle, adapted from [40]

Argasids are usually found in arid, high temperature areas with tropical and temperate climates [39]. Leathery cuticle, a special feature of soft ticks, allows them to survive unfavourable conditions by reducing the water loss [36]. Nevertheless, they are living in sheltered habitats such as caves, crevices of woods and walls, burrows as well as soil surfaces under the rocks covered with sand or dust. Most species are nidicolous, feeding on hosts that irregularly visit nesting areas for a limited time only. Consequently, they can survive substantial periods of starvation, and therefore prolong their life cycle for many years [37, 39, 41].

Moreover, soft ticks are characterized by multi-host life cycle [34, 42]. They acquire infection upon feeding on a host infected with *Borrelia*. Within few hours after tick's attachment, spirochetes migrate and establish infection in the midgut and salivary glands. Subsequent transmission of pathogens occurs during blood-meal on another host [38, 43, 44].

### 1.5.1. *Ornithodoros* ticks

Upon an infection, *Borrelia* within *Ornithodoros* ticks may persist for months, or even years [13, 45]. They are hematophagous organisms at each developmental stage [19].

*O.moubata* is an arthropod vector of various diseases identified in the eastern and southern regions of the African continent. These soft-bodied ticks are transmitting various infectious agents such as *B. duttonii*, a causative agent of Relapsing Fever, and African swine fever virus

[34, 35, 44]. Figure 3 shows a dorsal and a ventral view of *O. moubata* tick.

Figure 3. *Ornithodorous moubata*, adapted from [46]

## 1.6. Host-vector-pathogen interaction

RF *Borrelia* are spirochetes circulating in nature between their tick vector and a vertebrate host [5]. All of RF *Borrelia* species are kept within enzootic cycle with human as an incidental host with the exception of *B. duttonii* which has no other naturally identified reservoir than human [5,13]. The specificity of a vertebrate reservoir differs among species and include small mammals, usually rodents, human, birds as well as some wild mammals [2,13,19]. Therefore, mammals represent reservoirs of TBRF and the ticks' hosts. Additionally, arthropod tick vectors are sometimes regarded as original naturally occurring reservoirs of RF *Borrelia* species due to robust relationship between them and the bacteria [13, 45].

Due to numerous nymphal stages and blood meals, *Ornithodoros* ticks have many opportunities for transmission of RF *Borrelia* species [13]. As fast feeders, these ticks are attached to a vertebrate host for a short time but are still able to transmit pathogens even within seconds due to *Borrelia*'s preadaptation for the entry into a bloodstream of a mammalian host. Therefore, the spirochete population residing in salivary glands is of outmost importance for establishing the infection in a vertebrate host [28, 47]. Additionally, transmission of TBRF B*orrelia* species can occur through placenta and blood transfusion [19].

However, the interaction between *Ornithodoros* tick and a particular RF *Borrelia* species is defined by vector specificity. Even though some spirochetes can colonize ticks other than their unique vectors, the successive transmission to a host is not possible. Regarding the acquisition of spirochetes by argasid ticks, it was shown that *Borrelia* firstly colonize tick's midgut and subsequently migrate to the salivary glands [5]. Additionally, it was reported that *O. moubata* ticks produce antimicrobial peptides during infectious bloodmeal. Therefore, RF *Borrelia* had to develop a way to evade a vector immunity and cause a persistent infection within the ticks [48, 49].

## 1.7. Old-world tick-animal models

In order to investigate the interaction between the spirochetes, a vector and a host, different tick-animal models were proposed for the variety of RF *Borrelia* species.

Most studies carried out recently to examine the transmission and acquisition dynamics of RF *Borrelia* by its arthropod vector in the vertebrate host were achieved using the models developed with the new-world RF *Borrelia* species, namely *B. hermsii* and *B. turicatae* and their respective tick vectors *O. hermsii* and *O. turicata* [47, 50].

However, much less attention was concentrated on establishing the successful tick-animal model for the old-world RF *Borrelia*. The only functioning model which involved *B. duttonii* and *O. moubata* was developed using gerbils as hosts and it efficiently established both acquisition and transmission processes thus providing further evidence for preservation of this RF *Borrelia* species within the tick-human cycles [51].

Nevertheless, there is still a rising need for the development of a tick-animal model in other hosts to investigate different aspects of transmission cycles involving *B. duttonii* and *O. moubata*.

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