Acquisition Dynamics of *Borrelia duttonii* by *Ornithodoros moubata* over time

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Bachelor Thesis

Laboratory of Molecular Ecology of Vectors and Pathogens

INSTITUTE OF PARASITOLOGY

Biology Center, ASCR

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Bibliography:

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Annotation:

The aim of the thesis is to identify the transmission and acquisition dynamics of *Borrelia duttonii* by *Ornithodoros moubata* under normal as well as extreme conditions. Additionally, the infectivity of *B.duttonii* from brain tissue is tested to confirm data reporting on *B.duttonii* leaving a residual infection in the brains of mice.

Affirmation:

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only

České Budějovice, 14.12.2021

Signature: 

Abstract

Borrelia duttonii is one of many *Borrelia s*trains causing relapsing fever and the acquisition and transmission dynamics of the strain by *Ornithodoros moubata* is the main subject that was studied in this thesis. Furthermore, experiments were performed to confirm data which discovered the capabilities of *B.duttonii* causing residual infections in the brain of mice after the spirochetes were no longer detectable in the blood.

Experiments testing for acquisition and transmission dynamics used mice, infected by a standardized inoculation from a *B.duttonii* culture. After feeding new *O.moubata* periodically on the infectious mice, a time frame was established for the infectivity period of *B.duttonii* from a mouse reservoir. Post-inoculation, *O.moubata* can acquire the spirochetes from the host within the first month.

Further experiments tested the infection rate of *B.duttonii* by *O.moubata*, which were housed at different temperatures than the normal habitat conditions after they had acquired the spirochete from infected mice. The temperature changes did not affect the infectivity, presumably due to a high enough population of spirochetes within the vector to accommodate the possible loss of bacteria.

B.duttonii was additionally analyzed in mouse brains by homogenizing the brain and injecting the homogenate into naïve mice. The brain homogenate of some mice did appear to have Borrelia present to cause an infection in naïve mice. The occurrence was rare, and this could be due to the mouse strain used as well as the Borrelia strain .

These results provide further data in understanding the relapsing fever pathogen in its arthropod vector and the mammalian host.

Contents

1 Introduction

1.1 Relapsing Fever

Relapsing Fever (RF) is a disease caused by *Borrelia* which are gram-negative bacteria that are part of the *Spirochaetes* phylum. Some examples of known species which cause RF are *Borrelia hispanica, Borrelia recurrentis* and *Borrelia duttonii.* [1,2]

This disease is zoonotic which means that it is transmitted from a vector, such as an arthropod, to humans or other animals in a continuous cycle. Depending on the vector and the causative strain of RF, one Borrelia species can have one vector which can transmit the disease to one or multiple reservoirs in the wild. The majority of *Borrelia* species are transmitted through infected ticks, except for *B.recurrentis* which is the only species that can be acquired solely by the louse, *Pediculus humanus.* [3,4]

Due to the large number of RF Borrelia , different categories were made for tick-borne RF (TBRF) and louse-borne RF (LBRF). Additionally, TBRF is further subcategorized as soft tick-borne RF (STBRF) and hard tick-borne RF (HTBRF) depending on whether the vector is part of the *Argsidae* family (STBRF) or *Ixodidae* family (HTBRF). [4,5]

Regardless of the vector, RF shares common symptoms among all *Borrelia* species such as episodic fever, headache, hepatomegaly, myalgia and in some cases, as for an example an infection with *B.duttonii,* stillbirth can occur. In Africa, where *B.duttonii* and other *Borrelia* species are very prevalent, RF is often misdiagnosed as malaria due to the overlapping symptoms and shared geographical distribution. [1,6]

Additionally, the tools used to identify RF in patients are subpar thus further increasing the rate of misdiagnoses. Some current methods which are being used to identify RF are ELISA, Giemsa staining and analysis of blood smears under dark-field microscopy (see Figure 1). [7-9]

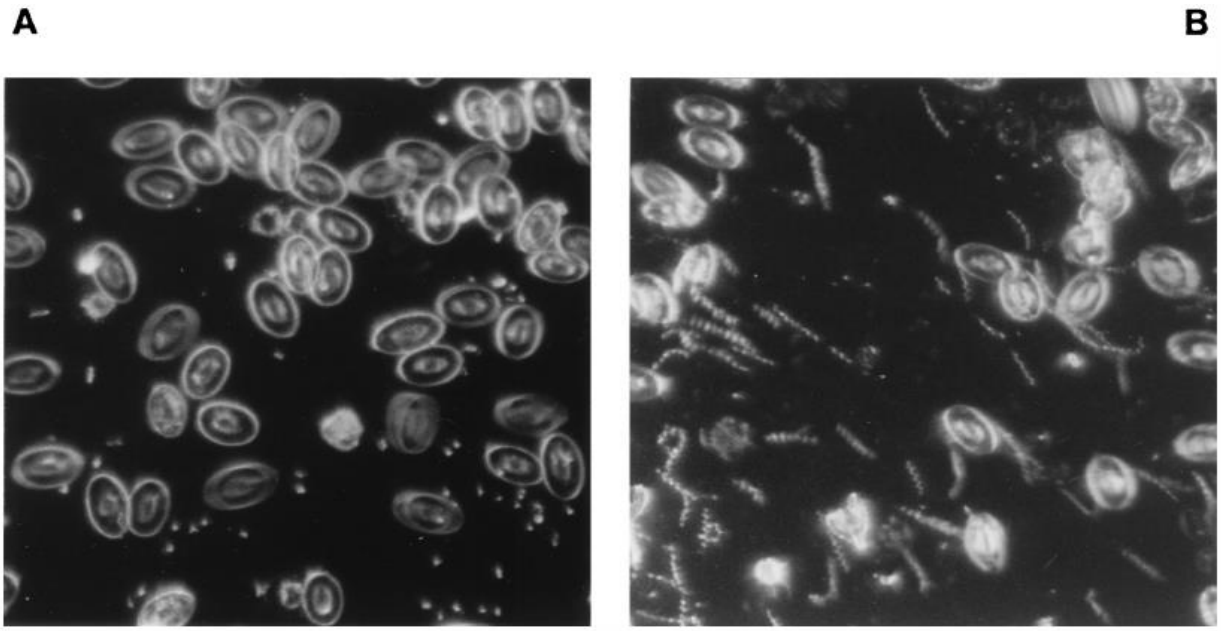

Figure 1. Picture A depicts blood cells under a dark-field microscope and B contains both blood cells and spirochetes, adapted from [10]

The limited number of diagnostics leads to mistreatment of RF with medication that is used for malaria or other diseases, thus resulting in a higher mortality rate. Due to the variety of *Borrelia* strains, the mortality rate of RF is approximated to reside between 2.3 and 5%. [1,6]
In comparison, *B.duttonii* has a mortality rate of 2.3% and since it can additionally lead to stillbirth it is even more dangerous for pregnant women, causing an increase of prenatal mortality to 44% and miscarriages to 48%. [4,1]

As of now, the disease is considered to be transient but some papers, such as Pathobiology of African relapsing fever *Borrelia* from Larsson, have found that *B.duttonii* could cause a brain infection even after the spirochetes are no longer detectable in the blood. Additionally, the hosts gene expression was identical to an uninfected animal which suggested that the hosts' immune system could not detect the *B.duttonii* spirochetes which retreated into the brain. [11]
Speculations can be made on whether *B.duttonii* could cause a new infection from within the brain but the specifics of *B.duttonii* spirochetes infectivity or its frequency of retreating into the cerebrum has not yet been researched well enough to give any well-developed theories.

1.2 *Argasidae* and *Ornithodoros moubata*

Ornithodoros moubata are soft ticks, also known as argasids which are part of the *Arthropoda* phylum, and predominantly occur in Africa. Members of the *Argasidae* family can transmit different strains of *Borrelia* depending on the species. [3,12]

An example of an *Ornithodoros* spreading more than one *Borrelia* species is the *Ornithodoros erraticus* tick. This vector can be a carrier for both *B.crocidurae* and *B.hispanica* which are distributed through the old world in areas such as western and northern Africa, as well as the Iberian Peninsula. Unlike *B.duttonii*, which only uses human reservoirs in the wild, *B.crocidurae* and *B.hispanica* transmit *Borrelia* to many different mammals like most other *Borrelia* species in the old world. The only other *Borrelia* species which relies solely on human reservoirs is *B.recurrentis* which is a louse-borne RF dispersed worldwide. [13]

Different argaside species are located in different parts of the world and are often times associated with the *Borrelia* species which they transmit predominantly, as can be seen in Figure 2. *O.moubata* is widespread in east and south Africa and is the only tick vector carrying *B.duttonii* spirochetes. [4]

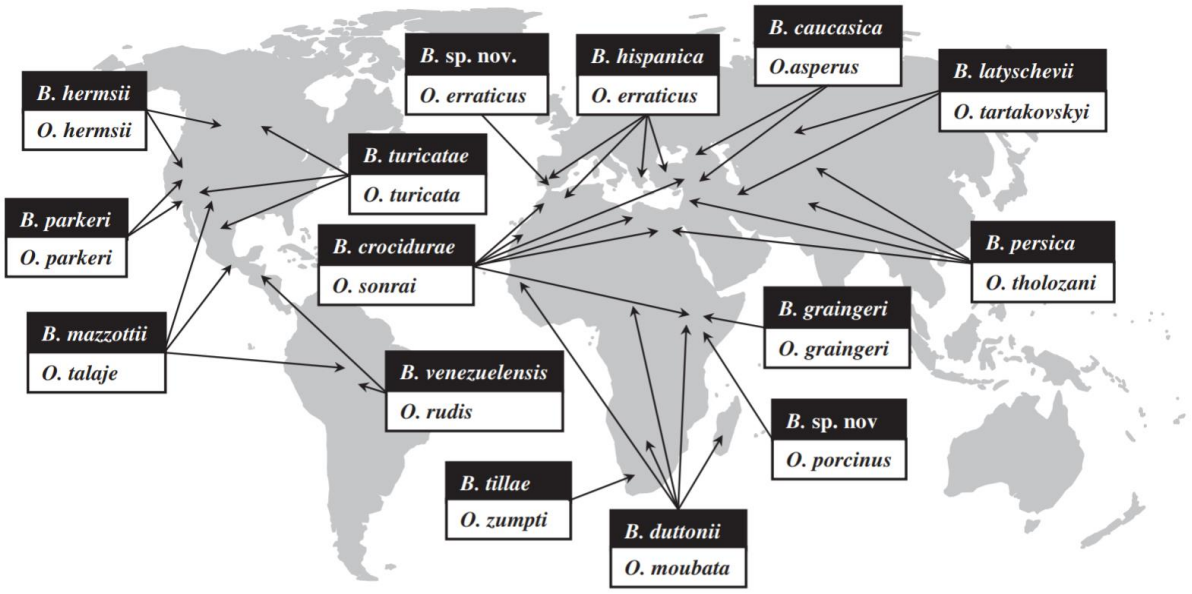

Figure 2. *Borrelia* strains and their argaside vectors across the globe, adapted from [4]

Even though argasids can be found globally, they are mainly distributed in climates that range from subtropical to arid. It is due to their unique morphology that soft ticks can survive very arid

conditions, which makes them better adapted to dryer climates than hard ticks (*Ixodidae* family). [3,12]

One of the physiological features that allows soft ticks to survive harsh environments is the integument. Unlike hard ticks, which have a hardened plate covering the dorsal area of their body, argasids have a wrinkled and leathery exoskeleton.
This unique integument will expand during a blood meal, allowing the tick to feed more rapidly and subsequently retreat quicker to a burrow. Furthermore, soft ticks can release excess water and ions via coxal glands during or after a blood meal which is used to protect the ticks' body from the environment. These physiological adaptations of the integument influenced the appearance of the mouthpiece of argasids as well. The mouthpiece, also known as the capitulum, is not visible from the posterior of an adult soft tick while it is clearly discernible in all life stages of an ixodid tick. [14,15]

Evolutionary traits, such as these, led to further changes in the life cycle of soft ticks. From one egg, a 6-legged larvae will hatch which can then moult to an 8-legged nymph regardless of whether a blood meal ensued in between. If feeding did occur, then the soft tick would withdraw into their habitats, which tend to be human shelters as well as cracks and crevices of rodent burrows. After retreating int o their territory, argaside ticks moult within a few weeks before leaving for another blood meal. [3,16]

Further on, the nymph can moult multiple times before it turns into an adult. After each moulting phase, which can either lead to a nymphal or an adult stage, a new blood meal is needed. When female nymphs grow into an adult, they can lay several eggs after each feeding session. A visualization of the life cycle can be seen in Figure 3. [16]

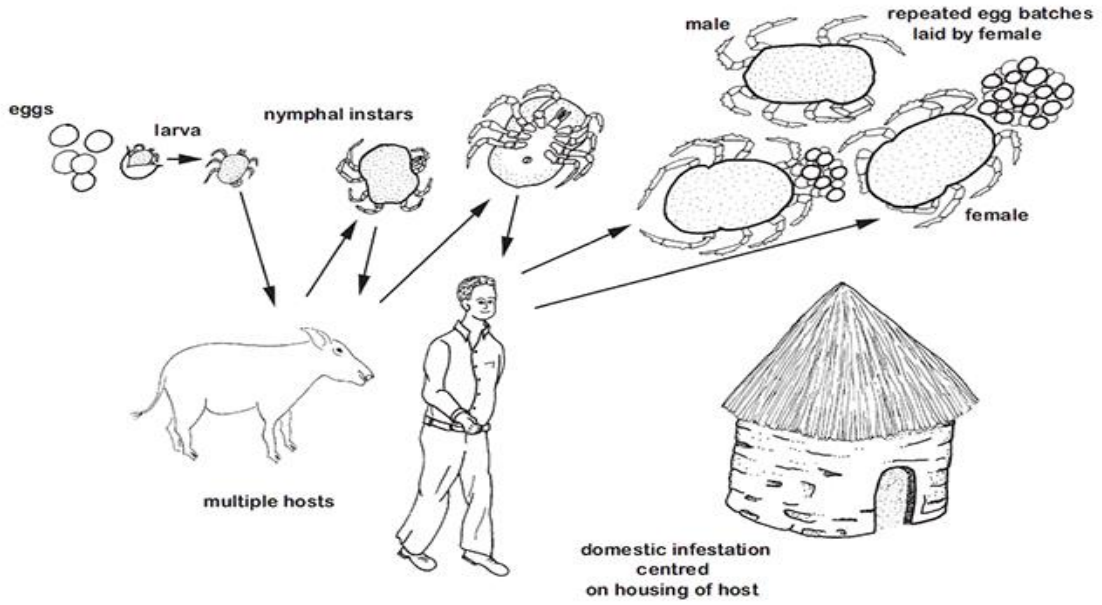

Figure 3. Life cycle of argaside ticks starting from the egg. A 6-legged larvae hatches which can feed on a host and moult into a nymph. After each blood meal the nymph can either moult into an adult or stay in the nymphal stage for a maximum of seven cycles. The female adult tick can then lay several eggs after each feeding, adapted from [17]

A stark difference between ixodid and argaside ticks becomes apparent in the duration of a blood meal. When argasids feed on a host, the time span of each blood meal is between 15 to 60 minutes depending on various factors such as the maturity and size of the tick. [18]

In contrast, hard ticks need to feed on one host for multiple days before they become fully engorged, and the time period is further influenced by whether the ticks are in the nymphal, or adult stage. Since soft ticks have a very short blood meal and moulting duration, it makes them optimal for scientific experimentation.

1.3 *Borrelia duttonii*

Borrelia duttonii is one of many *Borrelia* strains which causes RF and can be found in many parts of south Africa. Once *B.duttonii* is transmitted from the vector (*O.moubata*) to a human host, symptoms will generally appear between 4 to 18 days.

The episodic fevers are associated with the number of spirochetes circulating in the blood. During a fever, the number of *Borrelia* is higher than in the afebrile periods, which is due to the hosts' immune system combating the infection. Once the spirochetes are identified by the immune system, the infection decreases until the *Borrelia* evade the produced antibodies which

leads to another peak in spirochetemia, and a new febrile episode arises.

The spirochetes' evasive mechanism is possible due to antigenic variation of the RF *Borrelias'* variable major proteins (Vmps). The Vmps help RF spirochetes to be incompatible with the produced antibodies of the host, which is accomplished by varying the expression of their antigens. The variability of the Vmps stems from the two subcategories called variable large proteins (Vlps), and variable small proteins (Vsps). The major difference between Vlps and Vsps is the size of the proteins, and by changing the expression of these Vmps the spirochetes can hide from the hosts' immune system several times. The febrile and afebrile episodes can be seen in Figure 4 as well as a representation of how the Vmps change. [3,19-22]

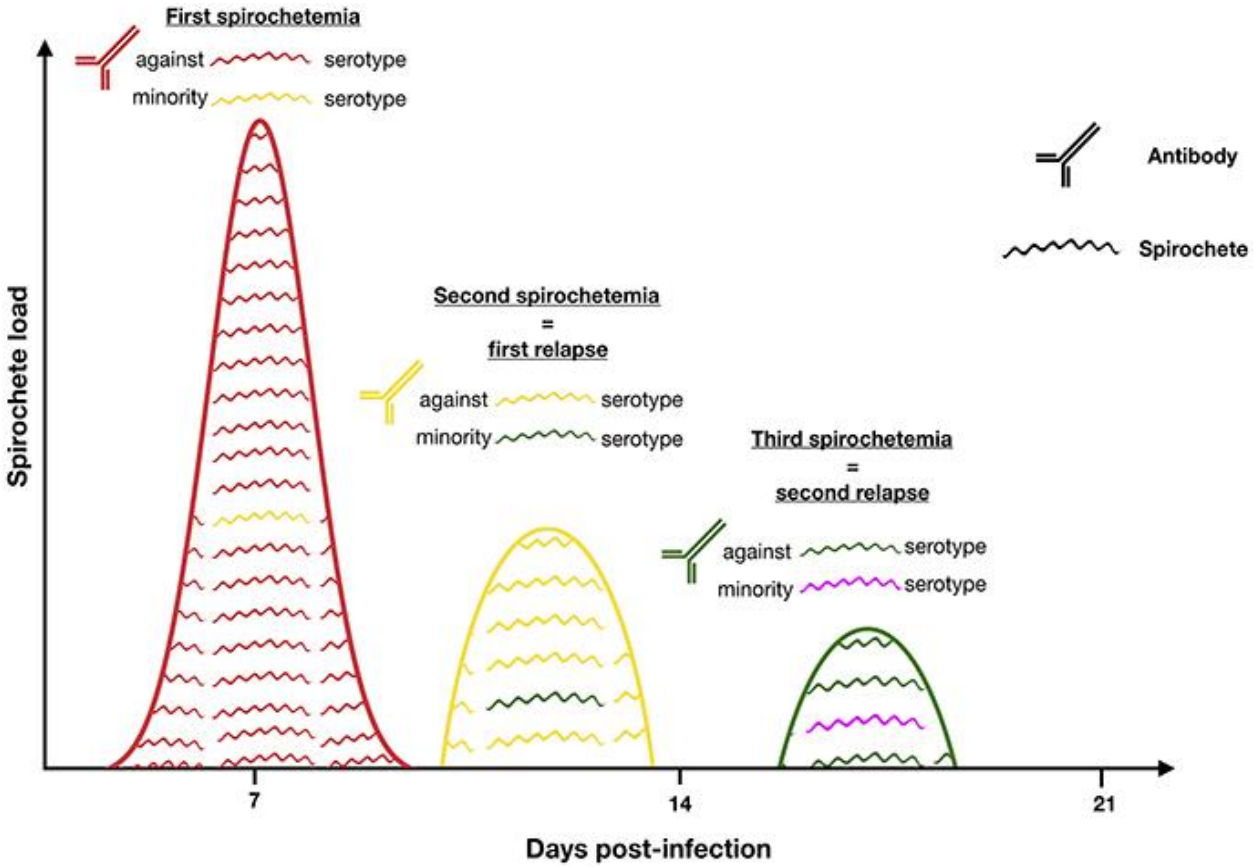

Figure 4. Graph depicting an overview of the evasive mechanism of spirochetes using Vmp expression changes, adapted from [5]

The number of times *Borrelia* can evade the hosts' immune system is termless. If the host cannot combat the infection, then the spirochetes will continue to multiply and change Vmps, as needed, until the reservoir is deceased.

As previously mentioned, *B.recurrentis* and *B.duttonii* are the only known species to exclusively use human reservoirs in the wild which could be due to the genetic similarity between the two *Borrelia* species. As can be seen in Figure 5, *B.recurrentis* and *B.duttonii* are phylogenetically related which is reflected in the Vmp sizes, G+C ratio and 16S rRNA resemblance. [4,5]

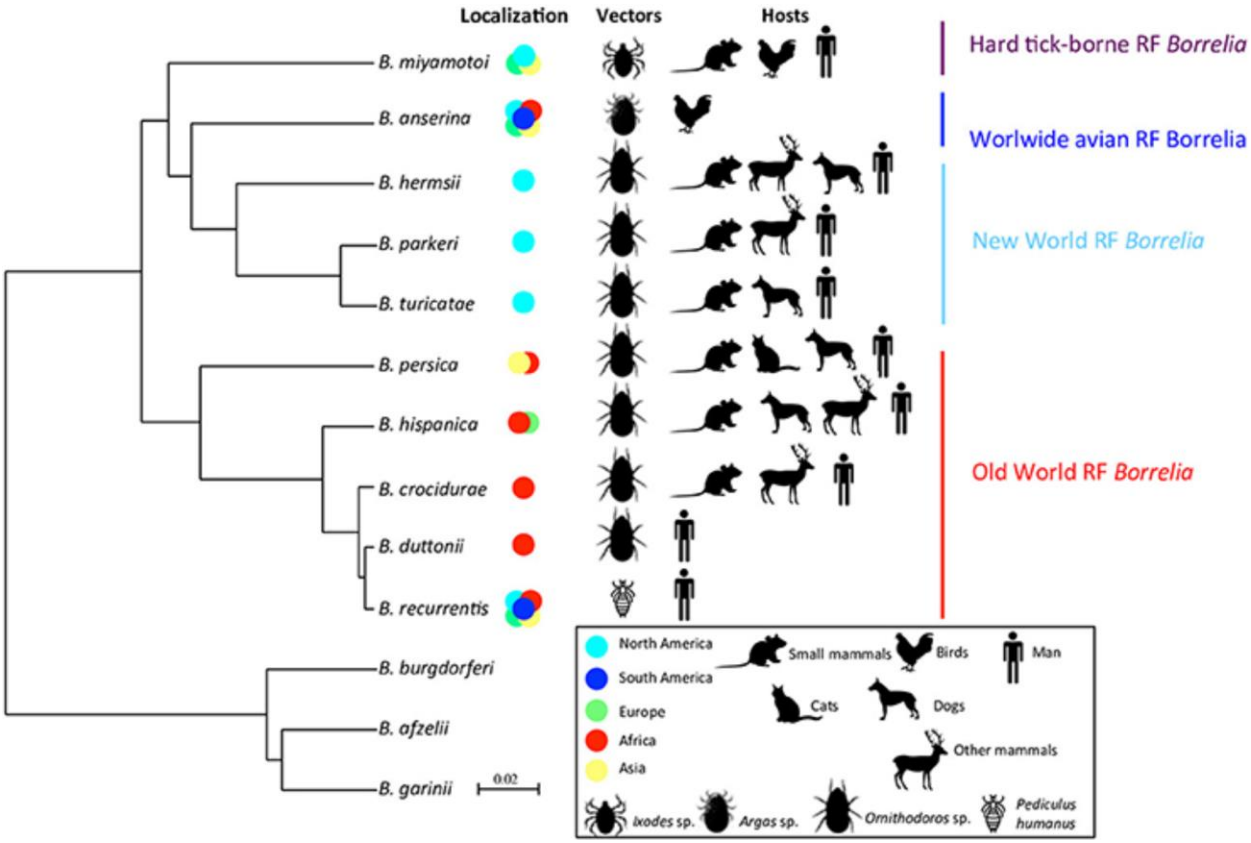

Figure 5. Depiction of *Borrelia* strains with their respective vectors and hosts as well as their global occurrence and phylogenetic correlation, adapted from [5]

Unlike other *Borrelia* species and their respective vectors, *B.duttonii* and *O.moubata* are neglected in many scientific experiments. There are a multitude of topics that still need to be analyzed as for example the acquisition and transmission dynamics of the vector. Furthermore, previously mentioned topics such as *B.duttonii* retreating into mice brain or the interchangeability of vectors between *B.recurrentis* and *B.duttonii* are yet to be further analyzed or experimented with at all.

Some earlier research has shown that *B.duttonii* can infect lab mice making it possible to experiment with this *Borrelia* species in the lab using mice models. [7]

Since mice are used as a substitute to human models, any research data acquired cannot be guaranteed to correspond completely to natural interactions like many other diseases which us substituted models for experimentation.

Previous lab experiments on acquisition dynamics of *B.duttonii* by *O.moubata* have identified the threshold of necessary spirochetes which is needed to cause an infection in the vector and subsequently in a mouse. The data has shown that once *Borrelia* are visible in the blood of the host the infectivity rate for the vectors is 100%. The observation was performed over 15 days, which leads to inquiries as to how long this infectivity rate lasts beyond the 2 weeks. [23]

Further research done on survivability of *O.moubata* infected by *B.duttonii* has shown that the infection rate of the ticks significantly decreased when the vector was housed at a temperature several degrees higher than their natural habitat climate. [24]

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