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Borrelia burgdorferi sensu lato and *Borrelia miyamotoi* in questing ticks and tick fauna of companion animals in East Germany.

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1. Introduction

Ticks are blood sucking arthropods, responsible for the transmission of a wide range of viral, bacterial as well as protozoan infectious agents. Not only humans might be affected but also domesticated and wild animal species. Tick-borne diseases are of increasing importance nowadays, not only in Germany but all around the world.

Lyme borreliosis (LB) is one of the most common tick-borne diseases with an increased incidence of infections amongst human beings. However, also other vector-borne diseases occur in an increasing number because of several ecological and epidemiological factors such as transportation of domestic animals. The latter fact is mainly due to increasing tick numbers that could be detected, mainly because of global warming, agricultural changes or other socioeconomic factors (Hartmann, 2013; Rizzoli et al. 2014).

Ticks represent a high risk for the health and wellbeing of humans and animals due to transmission of several pathogenic agents. Thus it is highly important to get information about the diversity of tick species and its potentially health-damaging transmitted diseases. The knowledge about certain tick-borne diseases is necessary to find out particular measures to avoid tick infestations and therefore an infection with the pathogenic agents itself (Hagedorn, 2013). Amongst domestic animals, dogs as well as grazing individuals represent the major risk group for requiring an infection with tick-borne diseases. Thus receiving information by identification of potential vectors in a particular area is highly important to get knowledge about tick-borne diseases and for their risk assessment. Determination of several tick species is mainly based on identification of adult females because the male ones are known to have less specific characteristics. The immature stages can often only be detected with the presence of adult individuals due to several difficulties. Studies about tick species and their distribution and approximate quantity will give us information about their biology as well as disease relationship (Hartmann, 2013).

For receiving that information about distribution and occurrence of different tick species as well as their infection rates with pathogenic agents in the eastern part of Germany, I divided my scientific work into two major parts. The occurrence of different tick species was analyzed by collection and finally identification of ticks from domestic carnivorous animals, mainly from dogs and cats. Additionally ticks were collected by the flagging method, were finally sent to a special laboratory in Bavaria and screened by DNA analyzing methods for possible infections with borrelial spirochetes. In my opinion it is important to receive an overview about possible risk factors in our daily life, to gain information about the epidemiology of certain vector-borne diseases. Therefore my scientific work also focuses on biology of ticks, their distribution throughout Germany and their potential for transmitting several infectious diseases. Additionally also two vector-borne diseases, LB as well as relapsing fever (RF), are explained in detail with special regard to Lyme disease in dogs.

2. Literature part

2. 1. Systematics of ticks

Ticks belong to phylum **Arthropoda,** further divided into the subphylum of the **Chelicerata**, arranged into the class **Arachnida**. The latter group is characterized mainly by tracheal respiration and a division of the body part, consisting of one prosoma and opisthosoma. Arachnids have six pairs of appendages, composed of one pair of chelicerae and one pair of pedipalps or palps, additionally to four pairs of legs (Hillyard, 1996).

The Arachnids are further divided into the subclasses of the **Acari**. The mites as well as the ticks belong to this group, characterized by an anterior body part called gnathosoma and a posterior section, the idiosoma (Oliver 1989).

The order **Ixodida** that represents the general group of ticks: characterized by being either obligatory or only temporary blood-sucking ectoparasites. The size of the adult body is highly dependent on the feeding status of the females, varying from one mm in an unfed status up to three cm when completely engorged. Additionally a toothed hypostome is present at the mouthpart that is usually visible from above. The order Ixodida comprises altogether three families: **Argasidae**, **Nuttaliellidae** as well as **Ixodidae**.

The **Ixodidae** or hard ticks are the dominant tick species which are mainly of veterinary, medical and public health importance with regard to their total number of approximately 700 various species. The Ixodidae are further classified into two major groups, the **Prostriata** and **Metastriata**, consisting of five subfamilies and 13 genera. In the Prostriata you can find the anal groove located anteriorly to the anus, however for the Metastriata the anal anal groove is situated posteriorly to it.

The **Argasidae** or soft ticks are composed of a total of five genera, including approximately 190 species. However the most significant soft ticks belong to two genera, *Ornithodoros* that comprises approximately 100 species and Argas including 56 species.

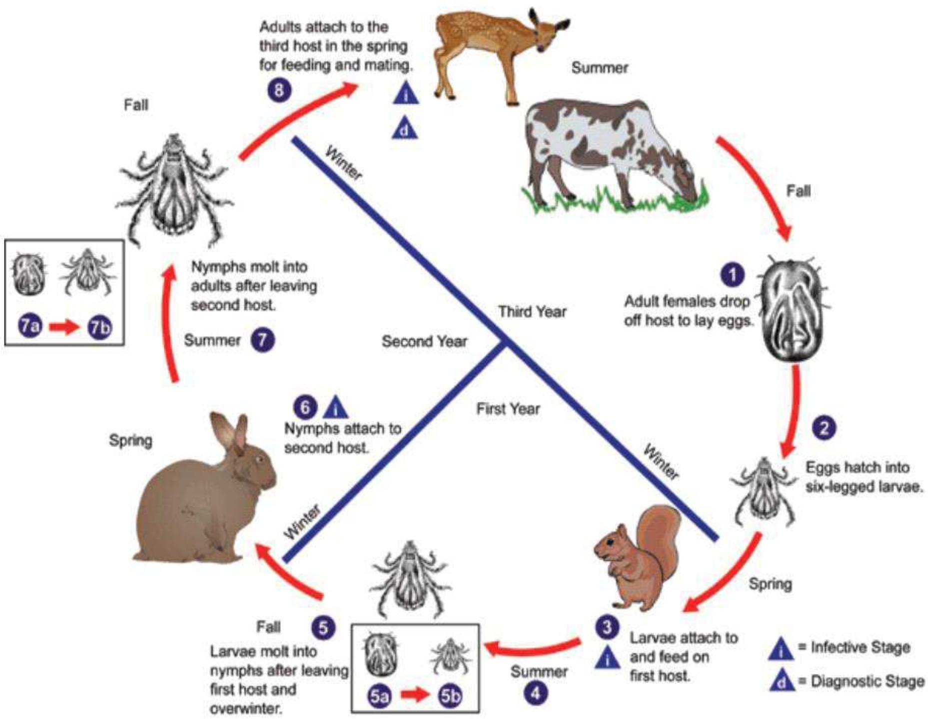
The last one, the **Nuttaliellidae** consists of a single species only, the *Nuttalliella namaqua* that can be found in the semiarid area of Namaqualand, South Africa (Oliver 1989).

2. 2. Reproduction and life cycles

The life cycle of ticks is composed of altogether four developmental stages, the eggs plus further three parasitic stages such as the larvae, nymphs as well as the adults.

The females become much bigger, even 100 times of their normal body size, when feeding on an appropriate host. Mating will occur on the host in case of all species that belong to genus *Ixodes*. After finishing the blood meal the female falls off the hosts and oviposition takes place, preferably in a hidden micro-environment. For the male ticks, except for those belonging to the genus *Ixodes*, a blood meal is required for initiation of the gonotrophic cycle. In contrary to the Prostriates in which mating may occur either on the host as well as in the environment, the metastriate ticks exclusively mate on the host, mainly whilst consuming the blood meal by the females. After a short preoviposition period the females start to lay thousands of eggs continuously. However in some tick species there is a morphogenetic diapause between the blood meal and the oviposition and egg laying will not occur immediately afterwards. In total more than the half of the body weight of an engorged female is converted into eggs and thus being of highest profitability amongst all arthropods.

The **three host** life cycle is the most commonly occurring way of development which might be completed, from the time of hatching of the larvae until the next generation of larval individuals, within less than one year. However this is highly dependent on climate factors and diapause that could delay either the further development of the ticks or their host seeking behavior as well as oviposition. Thus the duration for completion of the life cycle might be as much as three years or more years (Sonenshine, 1991).



2. 3. Host - Vector - Pathogen interaction

The following features have to be present when designating a tick as a vector for certain pathogens:
(1) The possibility for the tick becoming infected with the pathogenic agent whilst feeding on the infected host.
(2) The possibility of the engorged tick to transmit the pathogenic agent transstadially when molting to the next stage of the life cycle or to the F1 generation respectively.
(3) The possibility of the tick to infect the potential host with the pathogenic agent during blood feeding (Estrada-Peña et al. 2013).

In conclusion it has to be stated that for a designated vector, the tick must be able to feed on an infectious vertebrate and has to become infected during the blood meal. Furthermore it has to maintain the pathogenic agent via transstadial pathogen transmission and afterwards is able to infect new potential hosts whilst feeding again.

The local and regional appearance of certain tick species is highly dependent on the presence of suitable hosts, so called **'amplifying hosts'**, because only those are able to maintain the presence of particular tick species and a lack of these hosts will finally result in the absence of a tick species, even if the abiotic and weather conditions would be suitable for them.

Whether a host that is fed on by an infected vector becomes a **carrier** or a **non-carrier,** is dependent on a possible exposure to certain pathogens or not. However the carrier status does not necessarily mean that this host is infective for ticks. In conclusion to this the terms **reservoir** or **non-reservoir** have to be applied to determine the infectivity status. Regarding to this the non-reservoir host is not able to transmit certain pathogens to uninfected ticks.

The term **'host infectivity'** describes the efficiency of a host to transmit the pathogenic agents to further tick species feeding on it. The following terms such as **tick infectivity** and **specific tick infectivity** describe the efficiency of transmitting the infections from ticks to certain hosts, however the **specific tick infectivity** mainly refers to a specific tick species transmitting the infection to a specific host species (Estrada-Peña et al. 2013).

In general it has to be mentioned that the possible occurrence as well as its frequency of vector-borne diseases is highly dependent on the vector-pathogen-host interactions. In addition to this it is also influenced by certain abiotic as well biotic factors. Not only climate factors such as global warming affects spreading of certain tick species, additionally to the emergence of various vector-borne diseases, nor the increasing frequency of humans and domestic animals being exposed to ticks, also socio demographic factors, such as management derived alterations, e.g. deforestations or changes within the agricultural structure, are known to play an important role in recent changes in tick-host exposures as well as on tick infection rates. Thus screening for ticks infected by certain pathogens and the possible emergence of newly introduced vector-borne diseases becomes more and more important, especially in areas that are characterized by a high population density such as urban and suburban regions (Rizzoli et al. 2014).

2. 4. Tick - Borrelia Relationship

The *Borrelia* relationship is described in accordance to *I. scapularis*, the vector responsible for the transmission of LB in North America and various *Borrelia burgdorferi* s. l. genotypes.

Spirochetes are normally ingested by ticks during blood meal that immediately start to replicate within the midgut of various *Ixodes* ssp. After multiplication within the gut they start to migrate through the gut wall towards the haemocoel, following the invasion of the salivary glands. *B. burgdorferi* sensu stricto and *B. afzelli* are capable of downregulation of the outer surface protein A (OspA) whilst up regulating the OspC during feeding on the tick (Gray et al. 2002; Krupka & Straubinger 2010). Additionally it was described that an increase in temperature as well as the process of tick feeding itself facilitates the alteration of the antigenic structure in the outer surface protein profile which plays a significant role in infecting several mammalian hosts by these pathogenic agents (Schwan et al. 1995).

The previously described mechanism in changing the outer surface protein profile of several *Borrelia* genotypes enables those spirochetes to pass from the midgut to the salivary gland. It is described that the OspA is responsible for binding of the spirochetes to the midgut of the ticks which means that any factors that limit the up regulation of the OspC will result in reduced migration from the gut towards the salivary glands. The longer the feeding time of the tick, the more spirochetes can be found to appear within the salivary gland. However the time between tick attachments until transmission of the pathogenic agents may vary in respect to the different vector species as well as between several *B. burgdorferi* s. l. genotypes.

Thus the time required for inducing an infection within the potential host species may differ but is usually described by means of 24-53 hours after tick attachment and inoculation (Gray et al. 2002).
It is also described that not only the changes of the antigenic structure of *B. burgdorferi* sensu lato genotypes is responsible for successful tick infection but also immunosuppressive factors that are present within the saliva of the ticks. The latter aspect plays an important role not only for transmission of various *Borrelia* ssp. but also for facilitating the infection with tick-borne encephalitis virus which is described as saliva-activated transmission (Nuttall et al. 2000).

2. 5. Tick species occurring in Germany

The following chapter there are listed those tick species that have been reported to be present within the Federal State of Germany, in respect to this particular attention should be drawn to those species that commonly affect dogs and other carnivorous animal species.

Prostriate tick species:

Ixodes ricinus (Linnaeus, 1758)

It is the most commonly occurring tick species, not only in Germany but in the whole European region as well as the most important vector concerning tick-borne diseases (Petney et al. 2012; Földvári et al. 2005/2007; Rizzoli et al. 2014; Hillyard 1996; Hagedorn 2013; Beck et al. 2014; Rubel et al. 2014), which probably has the highest veterinary, medical as well as public health significance because of the ability of providing a suitable vector for a wide range of bacteria, protozoa as well as viruses (Petney et al. 2012).

Figure 2. Picture of a questing *I. ricinus* female (http://www.biopix-foto.de/photos/jcs-ixodes-ricinus-33242.jpg ; Download: 20.04.2015).

It has a wide range of wild and domestic animals, including also humans (Hillyard, 1996). It is a three host tick species, characterized by each developmental stage feeding once on separate hosts (Hagedorn 2013). All developmental stages usually feed on medium-large sized mammals, e.g. onsheep, cattle, horse, dog and cat. However the nymph and larvae also attack small rodents, additionally to several bird species and lizards. Even infestations on reptiles by *I. ricinus* have been described in the past (Hillyard, 1996).

In many studies about tick infestations in dogs *I. ricinus* could be collected most frequently in comparison to other tick species occurring in Germany (Beck et al. 2014; Hagedorn 2013; Entzeroth et al. 2009; Duscher et al. 2013; Dautel et al. 2006). Thus acquiring information about this tick species and its importance concerning vector-borne diseases has revealed many scientific papers and studies within the last decades.

Ixodes hexagonus (Leach, 1815)

This tick species can be found in most of the countries throughout Europe, especially in the western parts. After *I. ricinus* it is the second commonly occurring tick species that exists not only in Europe but also in North African as well as in Southern Asian regions, similar to the distribution ranges of *I. ricinus* (Hillyard, 1996).

In Germany *I. hexagonus* has been described to appear in nearly every Federal State throughout Germany (Petney et al.2012), even in larger cities such as Berlin (Beck et al. 2014).

Ixodes hexagonus is characterized by a nidicolous behavior. Therefore it can be mainly found on animals that prefer to live in nests and borrows such as hedgehogs (its main host: *Erinaceus europeus*; Petney et al. 2012), foxes, polecats and other mustelids (Meyer-Kayser et al. 2012), additionally to carnivorous animal species, such as cats and dogs (Capári et al. 2012.; Hillyard, 1996; Dautel et al. 2006).

Ixodes canisuga (Johnston, 1849)

It can be found evenly distributed throughout Europe, including many parts of Germany, such as Bavaria, Baden-Württemberg, Brandenburg, Hesse, Rhineland- Palatinate, North Rhine-Westphalia, Mecklenburg-Western Pomerania, Thuringia, and Lower Saxony (Petney et al. 2012). Additionally to Europe this tick species can be also found eastwards to southern Asia (Hillyard, 1996).

This tick species is also characterized by a nidicolous behavior and can be found mainly feeding on foxes and badgers as well as on other mustelids (Meyer-Kayser at al. 2012, Srèter et al. 2003), however many studies described heavily infestations also on cats and dogs (Petney et al. 2012; Capári et al. 2012; Földvári & Farkas, 2005).

Ixodes rugicollis (Schulze and Schlottke, 1929)

This tick is known to infest mainly mustelids in the Palearctic. It has been reported to appear within several European countries such as France, Germany, Switzerland, Romania and Poland. It has been originally collected for the first time in Germany (Siuda et al. 2010), in larger cities such as Berlin, additional to the Federal States of Brandenburg and Saxony but with a lower prevalence and intensity of infection (Petney et al. 2012).

Ixodes rugicollis is mainly found feeding on mustelids such as martens and weasels but could be occasionally also removed from foxes, raccoon, badgers and polecats. *Ixodes rugicollis* is also characterized by a nidicolous behavior because it can be usually found on predatory animals that tend to live in burrows and shelters with lower humidity. Research activities in Poland have also revealed that it parasitizes dogs and cats. However despite of being present in some parts of Europe, it is not as well-known as the other already mentioned tick species, especially in concern to the possible transmission of vector-borne diseases (Siuda et al. 2010).

The following tick species belonging to the genus *Ixodes* have been also reported to appear within Germany, however their main hosts are usually several bird species, small rodents and insectivores, additionally to bats and certain reptile species.

Table 1. Diagram of tick species of the genus *Ixodes* in respect to their occurrence within the Federal State of Germany and their preferred hosts (Petney et al. 2012 / 2015).

Metastriate tick species:

<u>Genus Dermacentor</u>

Dermacentor marginatus (Sulzer, 1776)

This tick species has been reported mainly from the western parts of Europe such as Spain, France, Italy and Switzerland, additionally to some warmer central European areas such as Ukraine. Outside of Europe it has been detected in North Africa, Central Asia and western Sibiria. Within Germany it could be collected in more south-western parts such as in Baden-Würtemberg, Bavaria, Hesse; Rhineland-Palatinate and the Saarland (Petney et al. 2012; Rubel et al. 2014).

It is usually described to parasitize mainly larger mammalian species such as horse, deer, cattle and sheep. Additionally it could be removed from dogs and hares but was not detected as frequently feeding on humans. The larvae and nymphs prefer to attach to rodents (Petney et al. 2012).

Dermacentor reticulatus (Fabricius, 1794)

This tick species is widely distributed throughout Europe, including many countries such as France, Spain, Poland as well as further central, western and eastern European states.

In Germany *D. reticulatus* has been reported to become much more important within the last decades.

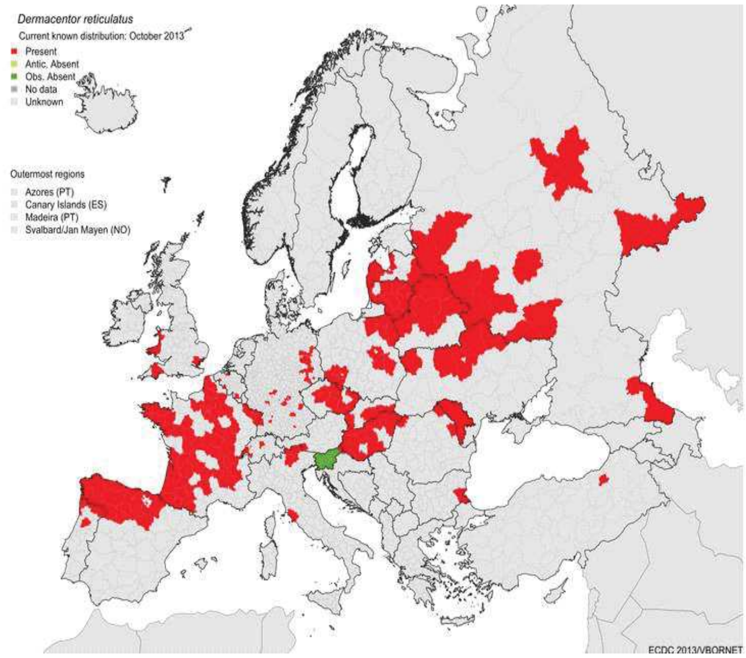


Figure 3. Map about the distribution of *D. reticulatus* within Europe (http://www.keneler.de/fileadmin/_processed_/csm_http___www.ecdc.europa.eu_en_healthtopics_vectors_tickmaps_ixodes-ricinus-map-distribution-high-res-october-2013_603fdd20b1.jpg; Download: 22.04.2015).

It has been found in several Federal States such as Baden-Württemberg, Rhineland-Palatinate, Brandenburg, Lower Saxony, Saxony, Saxony-Anhalt, Hesse and Bavaria which are described as typical *Dermacentor sites* (Dautel et al. 2006; Liebisch & Rahman 1976; Petney et al. 2012). It has been reported to become much more frequent not only in Germany but also in many other European countries such as Hungary, Slovakia and Poland (Petney et al. 2012).

The larval and nymphal stages parasitize mainly small mammals such as rodents and smaller carnivorous animal species, additionally to insectivores and occasionally also birds. However the adult stages prefer to feed on larger mammalians, including dogs (Petney et al. 2012; Földvári, 2005; Dautel et al. 2006).

Genus Haemaphysalis

Haemaphysalis concinna (Koch, 1844)

This tick species has been described to appear in European countries such as Romania, France, Germany, Poland, Austria, Hungary, Italy, Czech Republic and Slovakia with further reports outside of Europe from Asian countries like Russia, China and Japan.

Within Germany itself *H. concinna* was described to appear in Brandenburg, Hesse, Mecklenburg-Western Pomerania, Saxony, Saxony-Anhalt and Lower Saxony.

The larvae and nymphs prefer to parasitize mainly small-medium sized mammals such as rodents and hedgehogs. However the adult ticks can be mainly found feeding on preferably wild and domesticated ruminants such as cattle, deer, sheep and goat (Petney et al. 2012).

Haemaphysalis punctata (Canestrini and Fanzago, 1878)
This tick species can be found within the whole Palearctic regions, ranging from the United Kingdom up to Japan and from Scandinavian countries to Mediterranean areas such as Spain. Within Germany it was described to appear in Bavaria, Lower Saxony, North-Rhine Westphalia and Schleswig-Holstein.
The immature tick species can be mainly found feeding on small rodents, lizards, insectivores, hares and birds. The latter animal species is described to play an important role in transport of immature tick species during migration. The adult ticks prefer to feed on wild and domesticated ruminants such as cattle and deer, however they have been also reported to attach to humans.

Rhipicephalus sanguineus (Latreille, 1806)
This tick species is reported to occur worldwide from latitudes between 50º north and 30º south, however it can be mainly found in areas characterized by a Mediterranean climate such as the southern Europe or Africa.
Within Germany *R. sanguineus* is frequently introduced by dogs that have been returned from warmer climate areas. It was found in more southern areas within Germany such as Hesse, Baden - Württemberg , Bavaria but also in larger cities such as Hamburg and Berlin. High tick densities have been also reported from the city of Heidelberg (Gothe & Hamel, 1973).
Hosts: This tick species mainly parasitizes domestic dogs but occasionally could be also detected on hares, cattle and horses (Petney et al. 2012).

Occasional introductions
The following tick species that have been detected occasionally within Germany are only listed by their names within my scientific work because those were mainly introduced in very small numbers from other areas within Europe or North Africa by either migrating birds or by other animal species travelling with tourists such as *Dermacentor albipictus*; *Hyalomma lusitanicum*; *Hyalomma marginatum*; *Hyalomma rufipes*; *Ixodes caledonicus*; *Ixodes crenulatus*; *Ixodes festai*; *Ixodes persulcatus*; *Ixodes unicavatus*; *Ixodes ventalloi* and *Rhipicephalus microplus* (Petney et al. 2012).

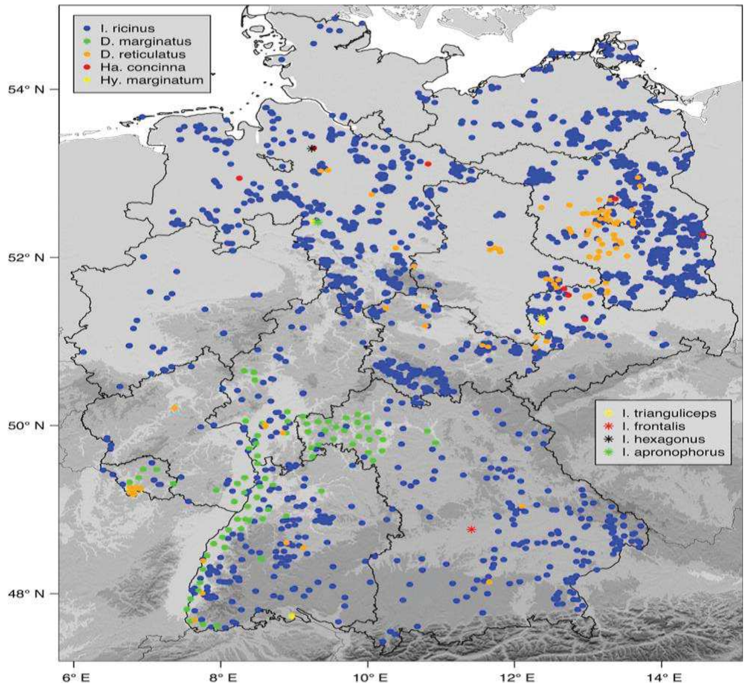


Figure 4. Map of georeferenced hard tick locations within the Federal State of Germany (Rubel et al. 2014).

2. 6. Tick-borne pathogens within Germany

Table 2 summarizes the most important tick transmitted pathogens according to those tick species that are the most predominant within Germany.

Table 2. Most commonly occurring tick species in Germany in respect to vector-borne pathogens that are transmitted by or detected in them (Petney et al. 2012; Schorn et al. 2011; Rizzoli et al. 2014; Erdélyi et al. 2013; Oehme et al. 2002; Schicht et al. 2012; Jongejan et al. 1999).

2. 7. Important features of Lyme borreliosis

Borrelia spirochetes are Gram negative, flagellated prokaryotes that are characterized by intense flexibility and a spiral shaped body-form (Földvári, 2005).

The genus of *Borrelia* is composed of two major groups. The first one, in which the LB spirochetes belong to, causes disease mainly in humans and some other mammalian species. However not all of the species belonging to this group are pathogenic ones. The LB agents are widespread in Europe and North America and their principle vectors are hard ticks (Ixodidae). The other group of the genus *Borrelia* consists of the relapsing fever (RF) spirochetes that are mainly transmitted by soft ticks (Argasidae). However some of them can be transmitted by hard ticks as well (Geller et al. 2012).

Lyme borreliosis, the most commonly reported tick-borne disease in humans in Europe and North America, is caused by spirochetes of the *Borrelia burgdorferi* genospecies complex. In general it is transmitted by hard ticks belonging to the genus *Ixodes*. In Europe it is transmitted mainly by *Ixodes ricinus*, with overlapping areas to *I. persulcatus* in more eastern parts. Twenty different genotypes of *B. burgdorferi* s. l. complex have been described so far and nine of those have been reported to occur in Europe including the following genotypes: *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. spielmanii*, *B. bavariensis*, *B. valaisiana*, *B. lusitaniae*, *B. bissettii* and *B. kurtenbachii*. For the latter genotype the disease in humans has not been confirmed so far (Stanek et al. 2011; Briciu et al. 2014).

The disease has been first described in the mid 1970's in Old Lyme, Connecticut, USA. It was referred to as Lyme arthritis because several cases of rheumatoid arthritis have been described, especially in very young children, after being exposed to a tick bite.

Both, in Europe as well as in North America, clinical symptoms of the disease are quite similar. Only minor differences could be detected so far which is usually a result of the greater variety of certain genotypes that are known to induce disease in Europe. In the USA, *B. burgdorferi* sensu stricto is the only pathogenic genospecies that causes LB in susceptible individuals, rarely inducing clinical symptoms such as acrodermatitis chronica atrophicans as well as borrelial lymphocytoma. The latter two are well recognizable in human beings infected by Lyme borreliosis agents.

LB became compulsory notifiable in certain European countries such as Slovenia, United Kingdom or Ireland. Thus comparable data are available nowadays that have shown that there is an increasing incidence of LB cases from the western to the eastern parts of Europe (Stanek et al. 2011).

2. 8. Important features of relapsing fever

The group of the RF spirochetes includes the following species:
Borrelia theileri that are transmitted by ixodid ticks belonging to the *Rhipicephalus* species, causing infections mainly in large livestock. Another member of this group is *B. lonestari*, transmitted by *Amblyomma americanum* and induces infections mainly in deer and finally
B. miyamotoi. The latter one can be found in smaller numbers in ticks in Eurasia and North America. It has been isolated for the first time in Japan in 1995 from *I. persulcatus* and from the blood of mice (*Apodemus argenteus*). The genetic material of closely related species could be also detected in *I. scapularis* and *I. pacificus* in the USA. According to some scientific researches, *B. miyamotoi* could be also detected in *I. ricinus* tick species in parts of Europe such as Sweden, Germany, Hungary and the Netherlands (Szekeres et al. 2015).
It has been reported that the Asian group of *B. miyamotoi* exerts a pathogenic potential, inducing LB like symptoms and relapsing fever. Clinical cases have been described to appear in humans in more eastern parts of Asia such as Ural and Sibiria. The first human case was reported from Russia in 2011, however much recently it was described to cause disease also in the Netherlands as well as in the USA. The detection of the causative agent of the RF is difficult. The latter fact is probably due to the fact that there are serological cross-reactions with *B. burgdorferi* s. l. antigens during ELISA or due to varying pathogenicity within the several European genotypes of *B. miyamotoi*. To detect the further spread of the Asian genotype with *I. ricinus* westwards to Europe, is therefore a matter of urgency to analyze and additional studies need to focus on this aspect in the future (Geller et al. 2012; Crowder et al. 2014; Szekeres et al. 2015).

2. 9. Clinical Lyme-borreliosis in dogs and cats

In case of LB in dogs the first important point to be mentioned is that the exact time when an infection by a tick bite has been occurred cannot be stated properly because in most of the cases it is overlooked by the owner. However not all dogs develop a clinically apparent infection because of seroconversion and after that remaining asymptomatic. In infections that have been induced experimentally approximately 75% of infected dogs had developed a disease that was clinically apparent.

Main characteristics of an acquired infection are usually fever, lameness, general malaise, combined with enlargement and swelling of the regional lymph nodes. In most of the cases those first clinical signs are quite unspecific and are usually overlooked by the owner because of their disappearance after a few days.

The development of an erythema migrans that can be usually observed in humans has not been described for dogs so far (Krupka & Straubinger 2010).

Weeks to months after the initial infection clinical signs might be observable, restricted to the joints mainly. Therefore intermittent lameness starts to develop due to the dissemination of the *Borrelia* into the skin, joints and connective tissue that frequently results in a local inflammatory response combined with swelling and pain. Clinical signs of severe lameness usually last two to five days and can be diagnosed as a form of mono- or oligoarthritis.

The further progression of the disease is characterized by reoccurrence of the lameness, either by limping on the previously affected limb or by changing of it to the opposite limb after two or three weeks, additionally to a moderate increase in body temperature and painful sections whilst moving up- or downstairs (Littman et al. 2006). Additionally also glomerulonephritis, asymptomatic encephalitis as well as myocarditis and cardiac arrhythmias might be possible consequences of an acquired infection (Krupka & Straubinger 2010).

Interestingly there were no reports about cats becoming infected naturally by the LB agents, despite of being exposed to tick bites as well as representing a suitable host species for *Ixodes ricinus*.

In experimentally infected cats only mild bacteremia was observed. However when a tick bite has been occurred the cats started suffering from lameness, arthritis and meningitis combined with measurable antibody responses.

Cats do not react as sensitive as dogs to infections with the LB agents. The latter aspect is not completely analyzed nowadays but it is hypothesized neither that these animal species are not susceptible to spirochete dissemination nor that their immune system is able to overcome an infection before onset of clinical signs.

One of the most important prophylactic measures for prevention of tick infestations in dogs is the topical application of acaricides or repellents. Those are usually available in form of sprays, powders, spot-on preparations as well as collars and shampoos. Additionally daily observation of the dogs as well as regular and early tick removal is highly important to prevent an infection with the pathogenic agents (Krupka & Straubinger 2010).

3. Materials and methods

3. 1. Collection of ticks from the field

Ticks were collected in monthly intervals from March until September 2014. It was decided to search for ticks only from one area during the whole collection period to find out the most important parameters that probably influence the collection rate of the number of ticks in a limited time period such as the temperature, the daytime, the relative humidity or other special features. If the tick collection site has heterogeneous undergrowth, it does not allow standardised distance-based collection of ticks in pre-set transects (Szekeres et al. 2015). For this reason, tick density was measured as number of specimens caught per hour and per collecting person.

The study was carried out on the Schafberg, which is situated in the village of Niederkaina, belonging to the county town of Bautzen, East Saxony. This place was chosen because no other researches concerning the tick infections with *Borrelia* ssp. as well as the possible occurrence of several tick species was performed in the past (Landesuntersuchungsanstalt Sachsen, 2008).

The Schafberg can be found on a height of 167- 201m above mean sea level and the vegetation consists of mainly conifers and a few deciduous trees. It is quite popular for the dog owners to take their companion animals out for a walk. Furthermore the roe deer (*Capreolus capreolus*) can be found in high numbers in this area (Christian Schäfer, personal communication).

The collection method which was chosen is the so called flagging method. A cloth, preferably a white one, is fixed to a wooden stick and is pulled over the vegetation. The ticks that have attached to the cloth were removed carefully by small tweezers and finally stored in an Eppendorf tube containing 70% ethanol solution. The further identification and separation of the ticks according to their developmental stage and the species was performed in the laboratory of the Department for Parasitology and Zoology in Budapest.

Figure 5a. **Figure 20b.**

Figure 5a. Map of the German Federal State. The dark green area shows the Free State of Saxony (http://www.oberelbe.de, Download: 10.04.2015).

Figure 5b. Map of the Free State of Saxony. The red ring marks the rural district of Bautzen (http:// www.umwelt.sachsen.de, Download: 10.04.2015).

Every time a meteorological station (Weinberger Gmbh & CoKG, Mebus) was brought to the tick collection with which it was possible to measure the temperature in ºC, the relative humidity in % as well as the cloud cover during the time being out for collection. Any special features such as changing weather conditions during the day or other vertebrates that have appeared during the collection period were noted. It is important to mention that all the time a small fox terrier mix, an uncastrated male dog, was involved into the collection. Sometimes it was even possible to collect the ticks directly from his coat.

The collected ticks were stored in a refrigerator on 4°C until transporting the material to the Faculty of Veterinary Science in Budapest for identification.

Figure 6. Picture of the vegetation from the Schafberg in Niederkaina.

3. 2. Collection of ticks by veterinarians

From March until August 2014 veterinarians were involved into the study that are situated in the administrative district of Bautzen which altogether covers an area of 2390 km², located in the north-eastern part of the Free State of Saxony. It consists of 59 municipalities and the largest North-Southern extension of the rural district is 63 km, the largest West-Eastern extension is 65km. According to the geographical features it consists of mainly pine woods in the North, intensive economy in the middle part up to mixed and spruce forests in the South (http://de.wikipedia.org/wiki/Landkreis_Bautzen; Download: 22.03.2015).

A total of 15 veterinary practices and one dog parlor were involved into the study. The latter one and five of the 15 veterinary practices were located directly in the city of Bautzen. The rest is evenly distributed throughout the whole administrative district, including the larger towns of Kamenz, Hoyerswerda, Pulsnitz, Bischofswerda, Radeberg, Stolpen and further villages such as Ebendörfel, Königsbrück, Wilthen, Großharthau and Crostwitz (Fig. 7).

The materials that were needed for the collection by the veterinarians have been sent directly by the post, but the majority was brought to the veterinary practices personally. The material consisted of a number of Eppendorf tubes, containing 70% ethanol solution for the proper storage of the ticks. Furthermore, small stickers for the labeling of the several specimens and sheets of paper, that had to be filled in by the veterinarians. The latter one was composed of two parts. The first one was an introduction about the aim of the study and how to deal with the materials. In the second part a short questionnaire had to be filled in for every dog or cat where a tick infestation was present during the clinical examination of the patient (Tab. 3).

Due to the fact that the willingness for the collection of the ticks and the combined filling in of the questionnaire by the veterinarians was described as not too successful in the past, I tried to talk to nearly each practitioner personally (personal communication: Gabor Földvári). Furthermore it was promised to the veterinarians that everyone will receive a short summary about the most important results at the end of the study. This information could be used by each veterinarian to give a better understanding to the owner of the pets about the risk factors concerning tick infestations and its possible consequences of vector-borne diseases.

The questionnaires were picked up personally from the veterinary practices at the end of August and were finally transported to the laboratory of the Department for Parasitology and Zoology in Budapest.

The ticks collected by the veterinarians were identified and the total number of tick infestation on each pet, the species of the animal, the collection date, the hometown of the owner, as well as the tick species and in case of adult ticks their sexes were recorded.

Later on an Excel-table was filled in containing the most important data, including the aforementioned ones added by the veterinarians plus further information such as the presence of multiple affectedness, tick species and the total number collected from one dog or cat, as well as other special features that were conspicuous throughout the collection such as the development of an erythema migrans on a dog (observation by Dr. Dietrich Lügner).

Table 3. Questionnaire to be filled in by the veterinarians
(Földvári & Farkas 2005; Odgen et al. 2000).

3. 3. Identification key of ticks collected from the field and by veterinarians

A stereomicroscope was used for identification of the ticks and all the collected specimens from one tube were transferred into a larger Petri dish containing 70% ethanol solution. The flagged ticks from the field were separated according to their species and developmental stage. However in ticks collected by the veterinarians the species and the developmental stage was noticed as well but when multiple affectedness has occurred on one cat or dog the total amount of collected ticks remained within one single tube only.

The adult male and female ticks as well as the nymphs were identified according Hillyard (1996). The larvae however were classified according to the scientific paper of Nosek and Sixl (1974).

3. 4. Process of DNA extraction

The extraction of the flagged ticks has taken place in the molecular laboratory of the Department for Parasitology and Zoology in Budapest.

For detection of the causative agents of the LB and RF group, it was decided to use all occurring developmental stages, especially the adult specimens that have the major epidemiological role. According to this we used a total of 50 female, 67 male ticks and 510 nymphs and 849 larvae, all identified as *Ixodes ricinus*. The latter ones were arranged in pools because of their smaller size and higher number.

At the beginning of the DNA extraction process three Petri dishes were prepared. The first one contained 70% ethanol solution, the other two were filled up with distilled water only. The ticks were transferred into the Petri dishes one after another for proper cleaning. After this procedure the ticks were cut into two pieces in a mediosagittal direction, so that the salivary glands remain intact. For every single tick a new sterile blade was used to avoid possible contaminations from one to another individual. Each single tick was transferred into a dry and clean Eppendorf tube and 1.25% ammonium-hydroxide solution was added into each sample. After this procedure it was double checked whether the ticks were divided properly into two pieces. The Eppendorf tubes were labeled and finally placed onto heating plates for altogether one hour and heated up to 100 °C. During heating of the DNA it is important not to cause any damages to the genetic make-up by using temperatures above the required limit or by overextending the whole procedure.

The larvae were extracted by the so called pooling method. After the washing and drying process, altogether ten larvae were transferred into a special, round-bottom tube and crushed into small pieces by a round ended glass rod. After adding ammonium-hydroxide solution and heating the mixture onto special heating plates up to 100 °C, the DNA was stored in a refrigerator at -20 °C. Finally the DNA was sent directly to the German Reference Centre for Borreliosis in Oberschleißheim, Bavaria where the PCR analysis was performed.

3. 5. Process of Borrelia identification by various PCR methods

In the following part of this study only the most important features concerning the PCR analysis are explained because no personal attendance has been taken place in the laboratory work that was performed in Oberschleißheim, Bavaria. All the necessary data for the used methods were taken either from published articles in which the different PCR methods were explained in detail or were sent directly to the author by Dr. Volker Fingerle who coordinated the laboratory work in Oberschleißheim.

The following PCR methods were used for the *Borrelia* genotyping:

The real-time **p41 duplex PCR** was developed for simultaneous detection of both pathogenic agents, *B. miyamotoi* as well as for *B. burgdorferi* sensu lato. This PCR method is used for targeting the flagelin gene (flab; p41), situated on a locus which is normally used for routine diagnostic PCR.

Table 4. Sequences of primers and probes for p41 real-time PCR (Venczel et al. 2015).

The **hbb PCR** is a real-time polymerase chain reaction that is used to target a histone like protein (the hbb) gene of *Borrelia burgdorferi* s. l., which is composed of altogether 327 base pairs (Briciu et al. 2014).

Table 5. Primers and probes that were used in the hbb RT-PCR (Briciu et al. 2014).

The **OspA genotyping** is a PCR plus sequencing method in which the formerly extracted DNA of the tick is first subjected to a semi-nested PCR (Glatz et al. 2014). It is used to distinguish between five relevant *Borrelia burgdorferi* s. l. species that occur in Europe which are the following ones:

Borrelia burgdorferi sensu stricto *, B. afzelii, B. garinii, B. valaisiana* and *B. lusitaniae* (Michel et al. 2004).

Table 6. PCR primer used in the OspA semi-nested PCR analysis (Michel et al. 2004).

The aim is the amplification of an 818-base pair fragment that can be found on the so called OspA (Outer surface protein A) gen that was used to identify the various *Borrelia* genotypes (Glatz et al. 2014). This method is followed by the restriction fragment length polymorphism (RFLP) analysis. The latter one has one or two characteristic RFLP pattern for each of the five *Borrelia burgdorferi* s. l. species, except for *B. garinii* (Michel et al. 2004).

Multilocus sequence analysis is known to be a highly sensitive laboratory method for precise description of *Borrelia* strains as well as for genetic research activities.

Table 7. Primer sequences, size of amplicon, and fragment for MLST/ recG (Wang et al. 2014).

This analyzing method works by amplification and comparison of sequences of different housekeeping genes such as **recG** that was used in samples. The fragment length must be the same as those available at the MLST webpage (http://borrelia.mlst.net). Concerning each individual strain, their amount of alleles of the loci built up a line of eight numbers that match with the alleles of the strains. Therefore the allelic profile is responsible for defining the sequence type (Wang et al. 2014).

Table 8. Amplification of **glpQ** by PCR (Margos et al. 2014).

The further differentiation was done due to the fact that some PCR methods are much more sensitive and specific to certain *Borrelia* genotypes and that some of them might be only detected by one single genotyping method.

4. Results

4. 1. Ticks collected from the field

Within altogether seven months, from March to September 2014, a total number of 1851 ticks were collected by the flagging method from only one area in the administrative district of Bautzen. All of these ticks were identified as *Ixodes ricinus*. A total of 50 female, 67 male ticks, 863 nymphs and 873 larvae could be counted at the end of the collection period (Fig. 9).

In June, when only a very low number of ticks could be found, the relative humidity was only 44% and 51% respectively on both days. *Ixodes ricinus* starts its host seeking behavior usually on higher relative humidity levels (Gray et al. 2009). In July however, there was an increase of the relative humidity from 68% to 82%, combined with a falling temperature from 27.8 ºC to 24 ºC during the collection days that probably could be seen in connection with increasing tick density at the end of this month. In September also very high relative humidity levels could be measured varying from 86% to 69% when a high number of larvae could be found. Except for July, no further connections between the temperature and tick density could be drawn throughout the whole collection period from March to September 2014.

The number of *I. ricinus* nymphs was relatively high and this was the most dominant developmental stage that could be found throughout the whole study. The number of adult ticks was slightly higher at the beginning of the collection period.

The first appearance of larvae could be recorded in May 2014 with a further peak in September of the same year (Fig. 9).

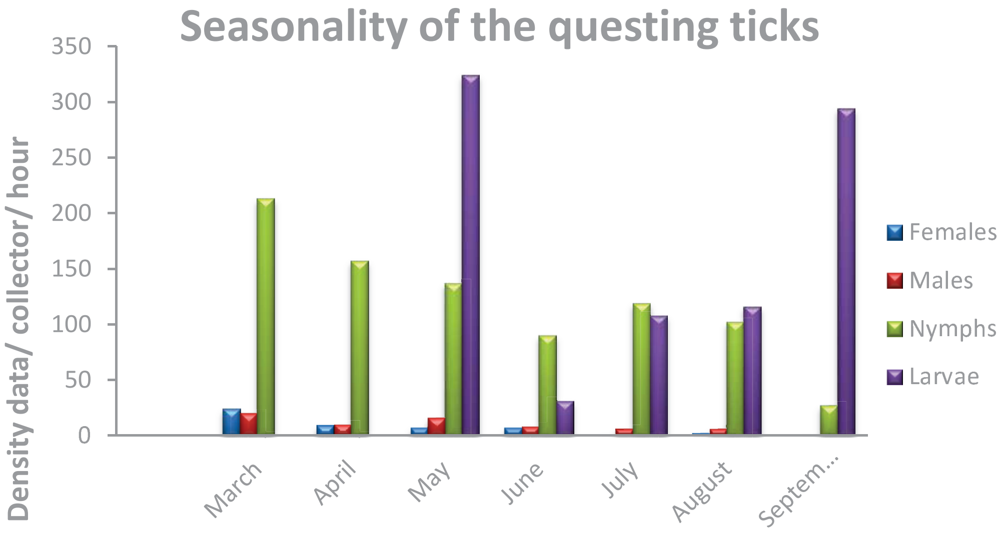


Figure 9. Bar chart about the seasonality of the questing ticks (March - September 2014).

It was also observable that there was a decline in the tick activity during collection in the summer months. However according to the density data there was a peak in September due to the high amount of larvae that could be found on these days (Fig. 9). The density of ticks during flagging ranges from 64 up to 150 ticks/ collector/ hour with a mean density of 93 individuals.

4. 2. Results of the PCR analysis

A total number of 1479 *Ixodes ricinus* ticks were used for the PCR analysis, composed of 50 females, 67 males, 510 nymphs and 849 larvae, the latter one being arranged in 10 larvae per pool. By the p41 duplex PCR method all tick specimens were screened for *Borrelia burgdorferi* s. l. and *Borrelia miyamotoi* infection respectively.

According to this a total number of 66 ticks were infected with *Borrelia burgdorferi* s. l. and 18 ticks carried *B. miyamotoi*. These positive samples were further analyzed for differentiation of the various genotypes, for *B. burgdorferi* s. l. according to the OspA semi-nested PCR method and the hbb RT-PCR analysis and for *B. miyamotoi* according to the recG PCR method and the glpQ PCR analysis.

The p41 duplex PCR method revealed that six tick specimens were infected with *B. afzelii*, four were analyzed by the OspA semi-nested PCR and the other two with the hbb RT-PCR method. Further two specimens were infected with *B. afzelii* together with *B. bavariensis* by the hbb RT PCR method, the latter one also revealed one positive specimen infected with *B. burgdorferi* sensu stricto. Further differentiation of the RF agents revealed the following results: for three specimens the further sequencing efforts were not possible and one specimen was positive for *B. miyamotoi* by the glpQ PCR method. By the recG PCR analysis in three cases the sequencing efforts revealed positive results for *B. miyamotoi* (Tab. 9).

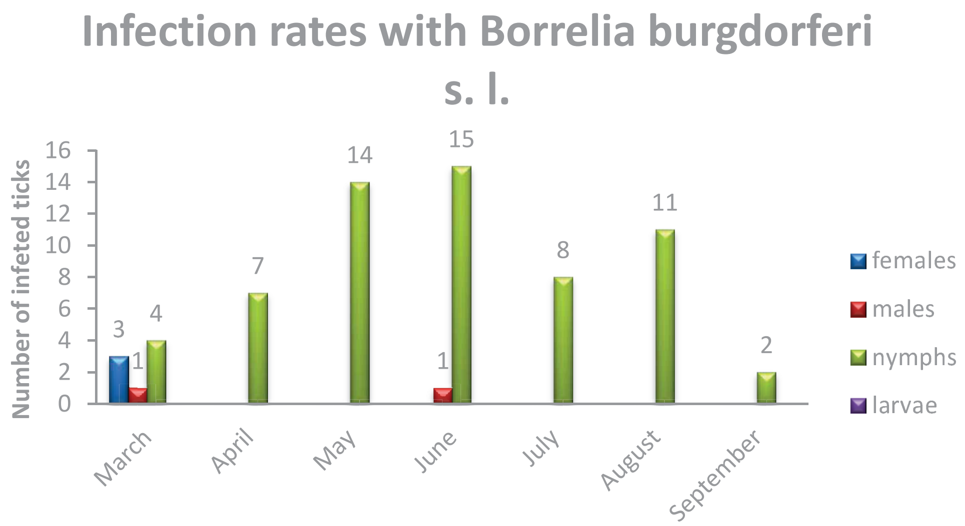


Figure 10. Bar chart about the infection rates with *Borrelia burgdorferi* s. l. in respect to the different developmental stages (March - September 2014).

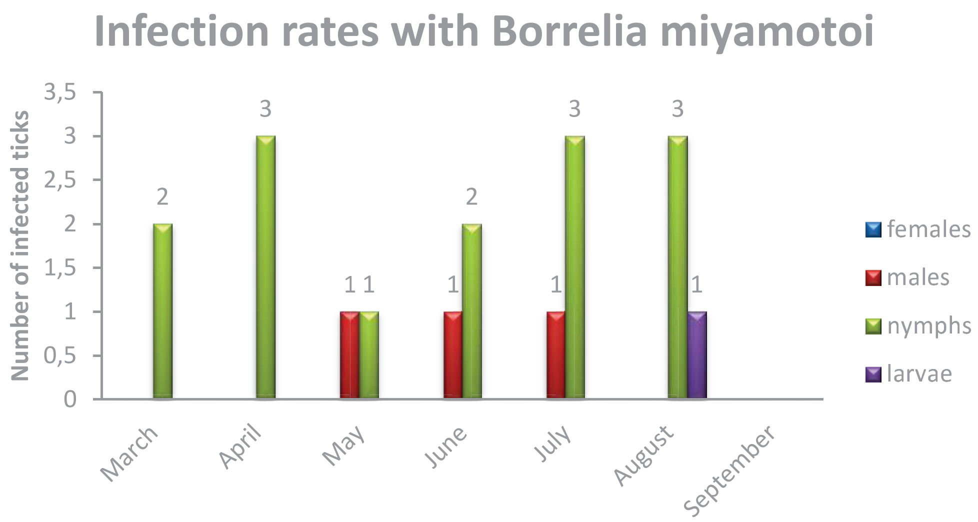


Figure 11. Bar chart about the infection rates with *Borrelia miyamotoi* in respect to the different developmental stages (March - September 2014).

Concerning the seasonality pattern the major infection rates could be analyzed during the summer months with a peak in June for *Borrelia burgdorferi* s. l. and in July for *Borrelia miyamotoi* (Fig. 10 & 11).

Table 9. Prevalence of *B. burgdorferi* s. l. and *B. miyamotoi* in questing ticks.

* = minimum prevalence

The nymphs were the most often infected developmental stage for both pathogenic agents that have been proven statistically by Fisher exact test ($p < 0.0001$). However, it has to be mentioned that they have occurred in a high number amongst the total number of the questing ticks and that the number of analyzed adults was quite low compared to the nymphs.

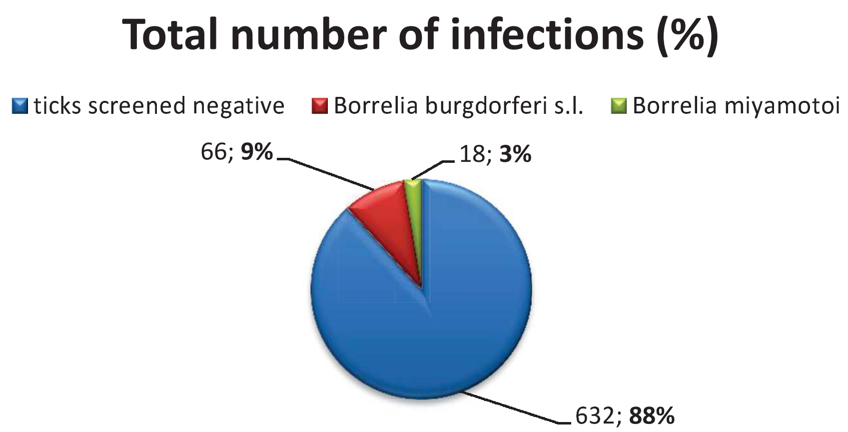


Figure 12. Pie chart about the total number of infections with the pathogens.

No pathogens could be isolated from the larvae in case of the LB agent. However for the RF group one single pool carried the *B. miyamotoi*, detected by the p41 duplex PCR method from the questing ticks of August 2014 (Tab. 9).

4. 3. Ticks collected by veterinarians

A total of 566 ticks were removed by the participating veterinarians at the end of the collection period from altogether 177 cats and 181 dogs, respectively. The dominant tick species that could be identified was *I. ricinus* which was found in 483 times of the infestation cases.

Ixodes hexagonus could be found 17 times evenly distributed on both dogs as well as on cats, followed by *I. canisuga* that was identified 11times. Only two *D. reticulatus* females could be found during the whole collection period, both feeding on dogs. In two cases both tick species, *I. ricinus* and *I. hexagonus* could be found on the same individual, once on a cat and once on a dog.

The most dominant tick species collected by the veterinarians from dogs and cats was *I. ricinus*. As well as for *D. reticulatus* also very low numbers of *I. hexagonus* and *I. canisuga* could be collected from the dogs and cats during the whole collection period by the veterinarians.

Concerning the frequency of ticks infesting either dogs or cats, it was quite equally distributed throughout the study. In total 177 cats (49%) and 181 dogs (51%) were infested by either one or more ticks.

However the intensity of infection was different. Cats were infested with a mean intensity of 2.25 and the dogs only by a value of 1.29 ticks/ individual. According to the Fisher exact test the intensity of tick infestations of cats is significantly higher than from dogs ($p < 0.0001$). The highest number was 12 *I. ricinus* specimen that could be collected from a single cat.

The developmental stage most frequently found on dogs and cats was the adult stage that could be found in 557 times of the infestation cases (97%), further divided into 505 female (86%) and 62 male (11%) ticks. The nymphal stage could be collected from the pets only 14 times (2%), followed by only three larval specimen (1%).

Significant higher infestations in cats were observed for the castrated males (39%, $p < 0.0001$), which was analyzed by the Fisher exact test. In dogs however the intact males were affected in a significant higher range (40%, $p < 0.0001$). For both species, ticks could be collected more often from male individuals than from the female ones, that has been also proven by statistical analyzes with the Fisher exact test ($p < 0.0001$).

The most commonly occurring attachment sites of the ticks on dogs and cats were the neck (36%), followed by the head (30%) and similar ranges of distribution was observable for the front extremities (13%), the abdomen/back (12%) and the hind extremities (9%).
The application of ectoparasiticides was performed regularly in 26% of the infestation cases. The same percentage could be observed for the pets that were never treated against ticks. In 48% of the cases the pets were treated, however the treatment was not as regular as the manufacturers of these products suggest it. The number of infested pets was not significantly higher in those that were never treated with ectoparasiticides compared to individuals treated regularly ($p > 0.05$). However, the rate of infestations in regularly treated individuals was significantly less than for those that were never treated which has been analyzed by the Fisher exact test ($p < 0.001$). Therefore the total number of removed ticks from only one individual and the route of application of ectoparasiticides, it has to be mentioned, that those individuals that were treated irregularly or never at all carried much more ticks at the same time than those treated in a regular way.

Table 10. Total number of removed ticks from dogs and cats in respect to the route of application of ectoparasiticides and multiple affectedness
(* multiple affectedness: ≥ 2 ticks on the same individual;
** total number of infested individuals with ≥ 2 ticks).

5. Discussion

5. 1. Ticks collected from the field

Only *I. ricinus* could be found by the flagging method in the area of the Schafberg. This is probably due to the fact that this region reflects the typical living space of *I. ricinus*, characterized by a moist microhabitat, a dense shrub layer (Hagedorn 2013), as well as a high amount of undergrowth and a broad humus layer. Another important factor for survival and further spreading of this tick species is the presence of appropriate hosts and its population density (Meyer-Kayser et al. 2012; Rizzoli et al. 2014). All of these characteristics concerning the vegetation and climate can be also found within the area of the Schafberg, as well as a high number of roe deer, cats and dogs from the surrounding residential districts, additionally to mice, voles, further rodent species and foxes. The latter aspect was also described by Gray (2002) who has described that the tick density during flagging is highly dependent on the climatic conditions and the presence of vertebrate hosts. In addition *I. ricinus* is the most commonly occurring tick species not only in this area of Germany but in the whole of Europe, as it was described previously in many other studies (Hagedorn, 2013; Entzeroth et al. 2009; Meyer-Kayser et al. 2012; Beck et al. 2014; Földvári, 2005; Rizzoli et al. 2014). Other tick species might occur as well. However their collection by flagging or dragging is quite difficult because most of them are endophilic ticks and could be found only in nests and holes, living together with their potential hosts (Meyer-Kayser et al. 2012).

At the beginning of the collection period the number of adult ticks was higher compared to the amount caught in the medium sections and at the end. This is probably due to the fact that this tick species enters the winter months preferably in an adult or nymphal stage and starts to seek for new hosts only by the rising temperatures at the beginning of spring and that the egg laying of the females will occur only after successful host seeking and blood feeding. In regard to this the first appearance of the larval stage could be recorded in May which shows that there were appropriate hosts available in the previous months. The same conclusion could be also drawn for the data in September when a further peak of the larval stage could be recorded.

There was a decline in the tick intensity during collection in the summer months (Fig. 9). However the number of the nymphal stage remained evenly distributed throughout the whole study in the field. This is also characteristic for *I. ricinus* that has its peak activity usually in spring (March - May), followed by another one in September and October (Beck et al. 2014; Hagedorn 2013; Duscher et al. 2013). During the summer months there is usually also a decline in the relative humidity level in connection with a rise in temperature. Therefore also the activity of *I. ricinus* is limited at this period within the year (Gray et al. 2009).

5. 2. Results of the PCR analysis

To the results of the PCR it has to be stated that the applied molecular methods could detect LB and RF spirochetes with high sensitivity as well as specificity.

The highest pathogen prevalence was observed during the summer months with a peak in June for *Borrelia burgdorferi* s. l. and in July for *B. miyamotoi* (Fig. 10 & 11). This higher number of pathogens is probably due to the fact that there are sufficient amounts of hosts available in summer for maintenance of the *Borrelia* ssp. and thus inducing the further spreading of the pathogenic agents.

Concerning the tick infections with respect to the different developmental stages the nymphs were most commonly infected with the pathogenic agents. Regarding to this it has to be mentioned that they have appeared in a very high number during the collection in the field and consequently a large amount of them was sent to the German Reference Centre for Borreliosis, Oberschleißheim. However no pathogenic agents of the LB group could be detected from the larval pools by the various PCR methods. In contrary to this for the RF agent one single pool was screened positive for *B. miyamotoi*.

In case of the LB group there is the widely accepted hypothesis that transovarial transmission does not exist during egg laying of the females (Krupka & Straubinger 2010), however for the causative agents of the relapsing fever group transovarial transmission seems to be possible (Crowder et al. 2014). The latter aspect is of high health importance concerning tick-borne infectious diseases because faster spread and distribution of the pathogens is possible when the causative agents are transmitted directly to the offspring.

The screening for the pathogens of the LB and RF group revealed that there is not a very high pathogen prevalence within the ticks in this area of Germany (Fig. 10 & 11), however *Borrelia* ssp. are still present and nevertheless there is a risk for humans and other mammalian species for becoming infected with the LB and RF agents which has a great public health impact. Another very important point in this study is the presence of the causative agents of the RF group that has never been analyzed prior to this study in the Eastern parts of Germany. When similar studies were performed in southern parts of Germany higher infection rates in ticks could be detected. From altogether 2155 examined *Ixodes ricinus* ticks, 475 of them were harboring *Borrelia* ssp. which is a higher infection rate than those detected in my scientific study. From those infected ticks the most commonly occurring *Borrelia burgdorferi* sensu lato species was *B. garinii* (34,3%) with minor percentages for *B. afzelii* (25.1%),

B. burgdorferi sensu stricto (22.0%), followed by *B. valaisiana* (12.7%). The least infection rates could be detected for *B. spielmanii* (5.9%) from 475 infected ticks (Fingerle et al. 2008).

5. 3. Ticks collected by the veterinarians

Concerning the distribution rates of the various tick species it was expected that *I. ricinus* could be found in high numbers feeding on cats and dogs within this area of Germany because former studies revealed the same results in similar studies when collecting ticks from animals (Entzeroth et al. 2009; Dautel et al.2006; Beck et al. 2014; Duscher et al. 2013; Beichel et al. 1996).

The small number of ticks collected in March 2014 is probably due to the fact that the questionnaires were only handed over to the veterinarians at the end of this month, so there was not enough time to collect that much ticks from the dogs and cats. Furthermore, it was observable that there was a peak of infestation in April which matches with the peak activity of *I. ricinus* within the year (Duscher et al. 2013; Gray et al. 2009; Beck et al. 2014; Hagedorn 2013). The steady decline that was observable throughout the collection period by the veterinarians from April to August is also common for this tick species and its usual way of host seeking behavior.

When similar studies were performed in more northern parts of Germany, in the Berlin/Brandenburg area, similar results concerning the tick infestation of dogs could be obtained. The majority was also *I. ricinus* (88,5%), followed *by D. reticulatus* (9,1%) and *I. hexagonus* (2,4%; Dautel et al. 2006). The reason why in this study only a few *D. reticulatus* could be collected is probably due to the fact that the adults are usually active from late August/ September until April/ May (Dautel et al. 2006; Beck et al. 2014). In the present study the collection period of veterinarians last only until the end of August, so finished at the time when *D. reticulatus* usually becomes active during the year. It should be also mentioned that the occurrence of this tick species has an endemic appearance, which is limited only to the so called *Dermacentor sites* that doesn`t include the very eastern parts of Germany (Dautel et al. 2006). Additionally, it was reported that
D. reticulatus has not only a high affinity to dogs but also to red deer that might serve as potential hosts (Dautel et al. 2006). However, in the area around the administrative district of Bautzen much more roe deer exist (Christian Schäfer, personal communication). The latter aspect could be also a possible cause for the much lower appearance of *D. reticulatus* in Eastern Germany. However no studies of ticks collected from deer had been performed in these parts in former times and therefore, further research would be necessary.
Despite of being present only in low numbers, *D. reticulatus* is still present in the very eastern parts of Saxony that might suggest further spread of this tick species within Germany including increasing the risk of pathogens being transmitted by *Dermacentor* species such as Canine babesiosis (Dautel et al. 2006).
Also very low numbers of *I. hexagonus* and *I. canisuga* could be collected from the dogs and cats. In former studies it was reported that their peak activity is usually from January until March and October until December respectively (Meyer-Kayser et al. 2012). In the recent study about the tick infestation in dogs and cats only the months in between were analyzed. That could be a possible cause for the lower number of *I. canisuga* and *I. hexagonus*. Furthermore it has to be mentioned that these two tick species have a much narrower host range in comparison to *I. ricinus*. They are characterized by a nidicolous behavior which means that they can be only found in caves and burrows of hedgehogs, foxes, polecats or other Mustelidae and only in particular cases dogs and cats serve as a host for them (Meyer-Kayser et al. 2012; Fölvári, 2007).
Special attention should be drawn to the tick infestation in cats. The most commonly occurring tick species, same as for the dogs, was *I. ricinus*, however finding the fox tick *I. canisuga* feeding on cats was rarely reported in the literature. Only Capari performed similar studies about tick infestations on cats in some parts of Europe in 2013. Not only *I. ricinus* could be found feeding in high numbers on cats but also *D. reticulatus*, *D. marginatus* and *I. hexagonus* in some eastern European countries such

as Austria, Slovakia, Slovenia and in Ukraine (Capári et al. 2013). Incidental findings of *I. canisuga* feeding on cats were also reported in several western European countries such as Germany, Denmark, France, Italy and the United Kingdom (Capári et al. 2013). Despite of some former studies, *I. canisuga* was detected not as frequently feeding on cats.

The most dominant developmental stage feeding on dogs and cats was the adult one. This is probably due to the fact that the immature stages do not necessarily feed on larger individuals such as dogs and cats. In most of the cases nymphs as well as larvae could be detected on rodents, birds or similar individuals with smaller body sizes. Furthermore it has to be mentioned that the immature stages are easily overlooked when checking our pets for possible tick infestations. Similar studies concerning the distribution of the different developmental stages on dogs revealed the same results (Beck et al. 2014; Földvári, 2005/2007; Duscher et al. 2013; Beichel et al. 1996).

Additionally to the high amount of different tick species feeding also on cats, it has to be mentioned that the intensity of the total number of ticks feeding on one feline individual is significantly higher than compared to dogs which has been proven statistically by Fisher exact test ($p < 0.0001$). In the recent study it was found that cats are usually carrying more than one tick at the same time. The reason for this phenomenon is probably due to the fact that cats remain much longer sitting within the vegetation due to their prey catching behavior. In respect to this care should be taken also for the feline species concerning tick-borne pathogens and vector transmitted diseases.

The most commonly affected attachment sites on dogs and cats were the head as well as the neck part. Thus screening our pets for possible tick infestations, special attention should be drawn to the front part of the animals 'body because of the higher incidence of becoming infested at these sites.

In my scientific work the infestation rates of castrated male cats and non-castrated male dogs was significantly higher than those detected in female individuals. Whether there exists a higher incidence of infestation within the different sexes of the pets has been analyzed in former studies but no special conclusion could be drawn because in these reports the ticks could be found evenly distributed on both, male as well as on female dogs (Beck et al. 2014). The reason for higher infestation rates in male individuals is probably due to the fact that their hormone levels are much higher than in females and that a high amount is excreted also by the urine.

The infestation intensity by the various tick species was much less in dogs and cats that were treated in a regular way compared to those treated rather irregular or not at all (Tab. 10). However no data exist about the used active ingredient of the product or which route of application was carried out on the pets. Furthermore, the used terms such as "regular, rather irregular or never" was not defined prior to the study and therefore was highly dependent on the veterinarians 'opinion.

This study revealed high and quite frequent tick infestations, not only in dogs but also in cats. According to this, there is an increased risk of acquiring infections by tick-borne pathogens within the area of the very eastern part of Saxony.

Concerning the geographical distribution of the various tick species it was quite evenly distributed throughout the whole administrative district of Bautzen. The two *Dermacentor reticulatus* females could be collected from dogs that were living near the border to Dresden. Former studies revealed high numbers of *Dermacentor* in and around the area of Dresden (Entzeroth et al. 2009). Also Dautel (2006) marked this area as a typical *Dermacentor site*.

Concerning the most commonly occurring attachment sites similar results could be also found in former studies when tick infestations on dogs were examined (Beck et al. 2014; Duscher et al. 2013; Földvári, 2005).

6. Conclusion

Nowadays tick-borne diseases are of increasing importance not only for humans but also for wild and domestic animal species. Thus it is necessary to understand the role of reservoir hosts, increased emergence of several tick species as well as potential hosts in which certain diseases could be induced. It is like a circuit in which several factors play an important role. Tick-borne infectious diseases are an emerging problem because of several factors that might act together in our modern world. Tick populations as well as their distribution ranges are changing steadily due to several factors such as climate changes like global warming, alterations in the agricultural structures and adaptation of ticks to cultural influences such as urbanization and increased travelling of people and their pets to all around the world.

With increasing tick numbers and steadily changing tick species, starting to appear in areas that were formerly free of certain parasitic species, there is increased risk of tick-borne diseases transmitted by them as well as the emergence of formerly unknown health threats for both, humans as well as for animals.

The aim of my scientific work therefore should focus on distribution ranges of several tick species, climate changes that possibly influences the activity of ticks throughout the year as well as host species that are most commonly infected. Additionally the role of transmission of certain pathogens has been inspected, such as LB spirochetes inducing Lyme disease in humans and dogs or RF agents affecting mainly the health and wellbeing of people. Thus ticks were screened for those emerging pathogenic agents to receive a picture about the recent situation in East Saxony, making statistical analyzes possible and inducing clarification of the risk of vector-borne diseases.

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