Attachment sites of different life stages of *Ixodes ricinus* ticks and infection of tick-borne pathogens in roe deer, badger, red fox and squirrel

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Abstract

As an effect of climate change and alterations in land use, the ranges of roe deer and also that of their ectoparasites, like the sheep tick (*Ixodes ricinus*) are expanding. This has also increased the occurrence of tick-borne pathogens that can be transmitted to humans and livestock, such as *Borrelia burgdorferi* sensu lato (s.l.) causing Lyme borreliosis in humans and *Anaplasma phagocytophilum* causing livestock fever in sheep and cattle. The impact of larger host animals on the transmission cycles for tick-borne pathogens has not been fully clarified. This study aims to contribute to add more knowledge on this subject by examining roe deer (*Capreolus capreolus*, n = 29), badgers (*Meles meles*, n = 14), red foxes (*Vulpes vulpes*, n = 6), and squirrels (*Sciurus vulgaris*, n = 17) for ticks. All data came from the municipality of Vestby, Moss and Frogn in Akershus (Viken, Norway) east of the Oslo fjord. I registered where ticks were located, in which stages of life, their number and on which animals they were attached. Tissue from all mammals were screened for the tick-borne pathogens *B. burgdorferi* s.l. and *A. phagocytophilum*. Roe deer had a prevalence of 100% for attached ticks. Adult ticks dominate on every part and especially on the back and the neck. Most of the nymphs are found on legs while larvae are mainly situated on head and legs. Roe deer had a high prevalence of 82% for *A. phagocytophilum*, but no *B. burgdorferi* s.l. infection. Badgers and red foxes had a prevalence for ticks of respectively 71% and 100% and with an intensity (ticks/individual) of 1.40 for badgers and 2.94 for red foxes. *B. burgdorferi* s.l. was found in both badger and red fox, and also a small amount of *A. phagocytophilum* with a 14% prevalence for badgers and 17% for red foxes for both pathogens. The red foxes had a high prevalence (69%) of dead, subcutaneous ticks. The squirrels had a high proportion of *B. burgdorferi* s.l., surprisingly no *A. phagocytophilum*. This makes the squirrels an efficient host for the transmission cycles for *B. burgdorferi* s.l.. My results show that the roe deer was the most competent host for in particular adult ticks, as evidenced from both prevalence (%), density and proportion of adult ticks, while squirrels mostly were an important host for nonadult ticks. Badgers seemed not significant for the tick's lifecycle. The high occurrence of dead, subcutaneous ticks in red fox indicate a low host competence. Other large-sized hosts thus seemed less competent hosts to ticks than roe deer. Hence, this provide further evidence that deer density is one key to limitation of tick-borne diseases, and hence of considerable management interest.

Contents

1 Introduction

There is an ongoing range shift of many species with current climate change and changes in land use (Parmesan 2006). This will affect management of many wild species. Looking into members of the Norwegian fauna, the roe deer (*Capreolus capreolus*) expand their range and density in a time span of only a few years, observable even for the common layperson. Similar range expansion can also be seen in their ectoparasites, such as deer keds (*Lipoptena cervi*) and the sheep tick (*Ixodes ricinus*). There is thus concern that infectious diseases spread by arthropod vectors will become more common (Jones et al. 2008). The direct effect of warming affecting positively the vectors are quite well documented, while the role of hosts for vector-borne diseases is less well understood.

I. ricinus is one of the most common ectoparasites in Europe. It is well known to be a vector for a number of pathogens such as *Borrelia burgdorferi* sensu lato (s.l.) causing Lyme disease in humans, *Anaplasma phagocytophilum* causing tick-borne fever in sheep, and *Babesia divergens* causing babesiosis in cattle (Mysterud et al. 2017). Studies reveal a shift in latitudinal and altitudinal distribution of *I. ricinus* in northern areas (Jore et al. 2011). The tick's life cycle includes four stages; egg, larvae, nymph and adult. Once it hatches, the tick is dependent on two blood meals to reach the adult stage; one meal from larvae to nymph and one from nymph to adult. Rodents and shrews are known to be the most important hosts for these early life stages, in particular larvae (Mysterud et al. 2019). In addition to this, the adult females need a large blood meal before completing the life cycle and lay their eggs (Mannelli et al. 2012). The adult ticks are therefore depending on large hosts for reproduction. However, there is little information on the extent to which large hosts may differ as hosts to ticks, and there are few studies on the selection of attachment sites on large hosts by different life stages.

The aim of the thesis is to increase the understanding of how important roe deer, badgers (*Meles meles*), red fox (*Vulpes vulpes*) and squirrels (*Sciurus vulgaris*) are as hosts to different life stages and species of ticks and as a reservoir of tick-borne pathogens. This will be achieved by:

- Quantifying *Ixodes ricinus* tick load on skin from roe deer, badger and red fox; number of larvae, nymphs, and adult ticks. Determine the spatial pattern of attachment on the body of the large sized hosts.
- Quantifying *Ixodes ricinus* tick load on body from squirrels; number of larvae, nymphs, and adult ticks.
- Quantifying load of all life stages of a more specialized tick species, *Ixodes hexagonus*, expected to be found on badgers and red fox only (Mehl).
- Quantify the infection prevalence of pathogens *Borrelia burgdorferi* s.l. and *A. phagocytophilum* in roe deer, badger, red fox and squirrel, expecting no *Borrelia burgdorferi* s.l. in roe deer and no *A. phagocytophilum* in red fox or badger, but possibly both in squirrel.

2 Material and methods

2.1 Study areas

Data derive from municipalities Vestby, Moss and Frogn in the landscape Akershus/Østfold in Viken county, Norway. This is located in the eastern part of southern Norway on the east side of the Oslo fjord south from Oslo. The area lies mainly within the boreonemoral vegetation zone and weak the oceanic vegetation section (Moen 1998). The average temperature through the year is between 4-5 °C in inner parts and assumed somewhat higher for the coastal areas. Annual precipitation at measuring station in Vestby, located at 75 m above sea level, is approx. 800 mm per year (www.met.no). The bedrock consists mainly of poor gneiss and mica slate, but in places exists some richer rocks indicated as gabbro and amphibolite. Within the Oslo field borders the rock dominates basalt, which is alkaline. Types of loose materials are bare rock and bare rock with thinner layers of loose material, marine deposits and marine beach deposits with a layer of sand and gravel over the more compact sea deposits.

On the steeper hills with thinner loose materials and acid bedrock east of the highway E6, it is evenly over poor vegetation. The areas are dominated by coniferous forest. The most common vegetation types are blueberry forest and small fern forest.

On the west side of the highway E6 towards the coast, there are plant communities that are more heat-demanding and which places greater demands on a richer bedrock. In the south-facing slope towards the village Son is a relatively rich plant life.

Typical landscapes from the central parts of Vestby where the majority of the data is from, are characterized by open cultural landscape used for agriculture with scattered ponds and there are mixed forest with for instance coarse oak (Blindheim and Olsen 2004).

2.2 Data

Data are road kills collected by the wildlife management authorities in the municipality. Sample sizes were 29 roe deer, 14 badgers and 6 red fox from 2015 to 2018. Further, there was sampled additional data of 17 squirrels in May 2016 gathered by hunting.

2.2 Preparing and collecting ticks from roe deer

The roe deer came either as whole carcasses or skins with their heads and feet attached. The process began with weighing the entire carcasses. To do this, they were hung up in a plastic bag on a digital hand-held scale, and the weight was measured and noted. The animals were stored at - 20 ° C and were therefore thawed for 24-48 hours at room temperature in the autopsy room.

Photo 1a-d: Whole carcasses of roe deer (a), badger (b), red fox (c) and squirrel (d) (photos by Christian Hügli).

The skin was stretched out on the autopsy table. The head was usually not skinned, although this was done on some received skins. The length from the base of the head in the neck to the base of the tail was measured and recorded. The attachment sites of interest were then cut from the skin and the area measured using graph paper. These parts were

(1) hind legs - from the hoof to the knee, thigh - the area between hips and knees
(2) belly – abdomen from hind leg to forelegs, cut in the middle to get front and back(rear) part (mostly to facilized the area measurement)
(3) forelegs - from hoof to knee
(4) shoulder - from knee to neck
(5) the neck
(6) a piece of the middle of the back, marginally adjacent to the neck.

Photo 2a-d: Parted skins from roe deer (a), badger (b), red fox (c) and a whole squirrel (d) ready to be screened for ticks (photos by Christian Hügli).

The parts were then searched one after the other for ticks. In order to find all ticks, the skin parts were shaved with a dog razor. This was achieved by two or more cuts in deepness with razor to avoid to destroy ticks and further to be able to find also the small larvae. Because of the origin of the carcasses, namely road kill, it was usually necessary to carefully dab off blood, tissue fluids and street dirt with an industrial towel so that shaving was possible. The firmly attached ticks were carefully removed with a brush. Only one side were searched, and which side was decided randomly with a coin. The same procedure was used for the head and one of the ears. The gathered ticks were identified to life stage (larvae, nymph, adult male and adult female), counted, and then placed in sample beakers with screw caps labelled with the animals' ID and the part which they were from and placed in a freezer at - 20 °C. The finished, processed skin parts inclusively head and ear were packed in freezer bags and stored for possible later controls.

2.3 Preparing and collecting ticks from badger

The badgers came as whole carcasses except two coming as skins with their heads and feet attached. The procedure was the same as with the roe deer with a few exceptions: both sides were examined, the legs included from claws to hip/neck, and the belly were counted as one piece.

2.4 Preparing and collecting ticks from red fox

All red foxes came as whole carcasses. The procedure was mainly the same as in badger. However, the right ear was missing and the abdomen was opened where a piece of the liver had been removed, as part of the Norwegian Veterinary Institute are investigating the presence of *Echinococcus multilocularis*. Therefore, it was difficult to process the skin with the shaver due to both dried and wet blood, and I chose the side with the least blood for examination.

2.5 Preparing and collecting ticks from squirrel

All squirrels came as whole carcasses. There was only one road kill. The others were sampled by shooting in the area of Son in May 2016 by an engineer at the CEES. A special permit was obtained from the Norwegian Environment Agency for shooting outside the hunting season.

The animals were stored at - 20 ° C and were therefore thawed over night at room temperature in the autopsy room. Again, the process began with weighing. For the squirrels a digital laboratory scale was used. Then the length from the base of the head in the neck to the base of the tail was measured and recorded. The whole body was examined and searched for ticks and shaving wasn't necessary. The picking and storage of the ticks was the same as with the other species.

2.6 Counting and classifying ticks

Lab work consisted of counting ticks on specific regions on each animal, and determine the species of ticks for badgers. The ticks were poured out of the cup on a white A4 sheet. At first, they were separated by size and then checked under stereo microscope to identify stage of development (larvae, nymph, adult) and for the adults their sex. They were counted and then put sorted by development stage in Eppendorf tube.

Photo 3a, b: Workplace for counting and classifying ticks (photos by Christian Hügli).

The life stages for *I. ricinus* have the following appearance:

A. Tick larva is smallest, often transparent and has only six legs.

B. Tick nymphs are usually bigger than the larva, but it depends on how much blood they have consumed. Nymphs have eight legs.

C. Adult males are usually bigger than the nymphs, have a “back shield” almost like a beetle, are dark in colour and have eight legs.

D. Adult females are biggest, have a dark body with a red abdomen and eight legs. When they are full of blood they swell up to the size of a pea and get a greyer look.

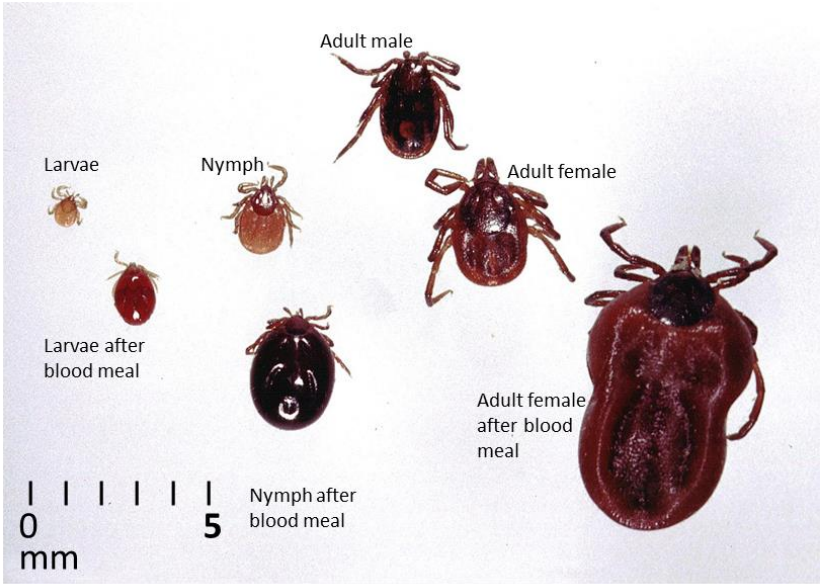


Photo 4: Stage of development for *Ixodes ricinus* (photo by Alan R Walker (https://commons.wikimedia.org/wiki/File:Ixodes-ricinus-life-cycle.jpg), „Ixodes-ricinus-life-cycle“, description by Christian Hügli, https://creativecommons.org/licenses/by-sa/3.0/legalcode).

Key features in differences between *I. hexagonus* and *I. ricinus* for adult ticks:
The legs and head parts of *I. hexagonus* are light-brown, while for *I. ricinus* they are dark-brown. The palps of *I. hexagonus* are shorter than the base of the gnathostome while for *I. ricinus* it is the other way round. The *I. hexagonus* has a distinguished hexagonal shaped brown scutum (shield) while for *I. ricinus* it is a rounder and black. The alloscutum (backer dorsal body) for *I. hexagonus* females is whitish and somewhat transparent so guts are shimming thru, while the marginal groove for both sexes is white to beige. Females of both tick species are light gray when they become swelled up as mentioned above.

Photo 5 a-c: (a) Head parts of *I. hexagonus* ("Ixodes hexagonus" by AJC1 is licensed under CC BY-SA 2.0), (b) Female *I. hexagonus* ("File:Ixodes hexagonus (aka).jpg" by André Karwath aka Aka is licensed under CC BY-SA 2.5), (c) Swollen female *I. hexagonus* ("Hedgehog Tick, Ixodes hexagonus" by AJC1 is licensed under CC BY-SA 2.0).

The Eppendorf tubes were marked by the sample name and development stage and then stored in a box in the freezer.

Lab work consisted of counting ticks on specific regions on each animal, and determine the species of ticks for badgers. A piece of tissue from the animal's ear was used to determine the presence of pathogens *B. burgdorferi* s.l. and *A. phagocytophilum* using an established rtPCR protocol (Mysterud et al. 2013).

2.7 Statistical analyses

All data were analysed with the statistic program R. A key part of the thesis was to provide descriptive information of prevalence, number and density of the different life stages of ticks per species and relevant body parts. This was presented as simple summary statistics in tables. I then merged some body parts to enable statistical comparison of the data from roe deer with the data from badgers and red foxes. Front and hind legs (left and right) and shoulder and thigh are all referred to as leg in that comparison. Belly, front and back (rear) and both sides go under belly. Head and ear go under head. Since the squirrels are counted as a whole, they cannot be used to compare the attachment sites.

I analysed variation in the number of ticks with a zero-inflated negative binomial model in the R-library "glmmTMB". The candidate variables were species (roe deer, badger and red fox) and body parts (back, belly, head, leg, neck). I compared 4 models with different factors and assessed their fit using the Akaike information criterion (AIC).

3 Results

3.1 Ticks on different host and on specific body parts

All of the collected roe deer had ticks attached so the prevalence was 100 % (n=29). No other tick species than *I. ricinus* were found. The adult ticks dominate on every part and especially on the back and the neck where they also are the most dense. Most of the nymphs were found on head and legs. Larvae were mainly situated on legs, and none were found on the back and the neck. Every body part had a prevalence of 100%. Highest density and intensity was on the back.

Table 1. Overview about parts with ticks on different hosts with tick prevalence, intensity, proportion of life stages and overall density (ticks per cm^2).

Species	Part	n	Prevalence (%)	Intensity (ticks per part)	Intensity (larvae per part)	Intensity (nymphs per part)	Intensity (adults per part)	Proption larvae	Proption nymphs	Proption adult	Density
Roe deer	all	29	100	8.85	0.22	2.35	6.27	0.03	0.27	0.71	0.028
	back	29	100	12.53	0.21	0	12.32	0.02	0	0.98	0.064
	belly	58	100	7.02	0.71	0.08	6.22	0.10	0.01	0.89	0.031
	head	58	100	9.82	4.62	0.02	5.18	0.47	0	0.53	NA
	leg	116	100	6.10	3.44	0.64	2.02	0.56	0.10	0.33	0.042
	neck	29	100	15.36	0.18	0	15.18	0.01	0	0.99	0.052
Badger	all	14	71.43	1.40	0	0.07	1.33	0	0.05	0.95	0.001
	back	14	7.14	1.00	0	0	1.00	0	0	1.00	0.016
	belly	28	17.86	1.00	0	0	1.00	0	0	1.00	0.006
	head	14	7.14	3.00	0	1.00	2.00	0	0.33	0.67	NA
	leg	56	7.14	1.00	0	0	1.00	0	0	1.00	0.012
	neck	14	21.43	2.00	0	0	2.00	0	0	1.00	0.014
Red fox	all	6	100	2.94	0	0	2.94	0	0	1.00	0.002
	back	6	33.33	2.50	0	0	2.50	0	0	1.00	0.008
	belly	12	25.00	1.00	0	0	1.00	0	0	1.00	0.010
	head	6	33.33	5.80	0	0	5.80	0	0	1.00	NA
	leg	24	12.50	1.33	0	0	1.33	0	0	1.00	0.005
	neck	6	33.33	2.25	0	0	2.25	0	0	1.00	0.014
Squirrel	whole	17	88.24	56.13	15.73	39.40	1.00	0.28	0.70	0.02	2.014

For the badgers the tick prevalence was 71% (n=14). All ticks were *I. ricinus*, as no other tick species were found. Only one nymph was found and was attached on the head, all others were adult ticks. Highest prevalence was on the neck with 21.43%, highest density was on the belly and highest intensity was on the head.

The red foxes had a tick prevalence of 100% (n=6). All ticks were *I. ricinus*, as no other tick species were found. Just adult ticks were found. Highest prevalence was on the neck with 33%, highest density was on the belly and highest intensity was on the head.

While skinning the red foxes I discovered subcutaneous adult ticks. They appeared from well-preserved ticks to moderate and advanced stages of decomposition. The prevalence for the red foxes for this phenomenon was 83%.

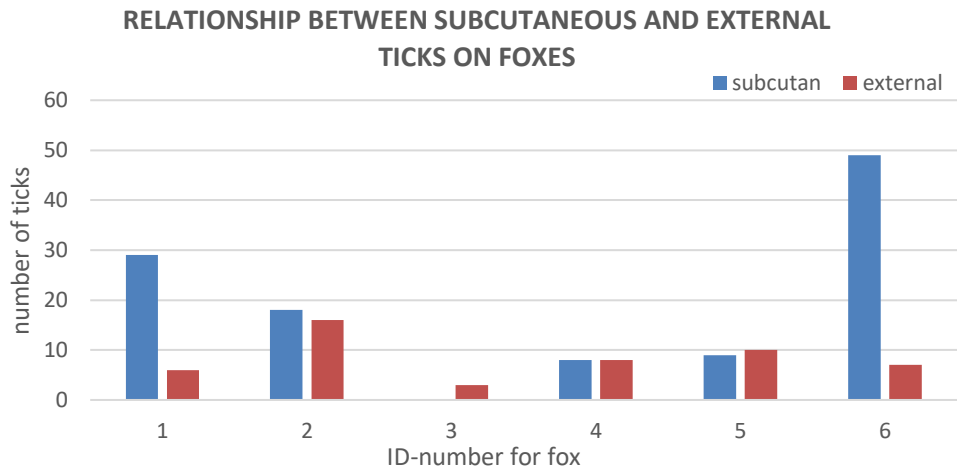


Figure 1: Visualization of relationship between subcutaneous and external ticks on red foxes. The y-axis shows the number of ticks found. On the x-axis are the individual red foxes.

The one with none found subcutaneous ticks had only three on its exterior although the red fox with the most subcutaneous ticks had non external ticks except on his head. The body parts with most subcutaneous ticks were belly and leg. 69% percent of all found tick were subcutaneous.

Table 2. Number of ticks found subcutaneous and external on specific body parts on the six red foxes. In total there are 113 subcutaneous and 50 external ticks found.

	RE001	RE002	RE003	RE004	RE005	RE006
subcutaneous ticks						
back	0	0	0	0	0	0
belly	3	5	0	0	0	8
head	NA	NA	NA	NA	NA	NA
leg	26	13	0	8	9	41
neck	0	0	0	0	0	0
external ticks						
back	1	4	0	0	0	0
belly	0	0	1	1	1	0
head	1	6	0	7	8	7
leg	3	1	0	0	0	0
neck	1	5	2	0	1	0

The squirrels have a *I. ricinus* prevalence of 88% (n=17). No other tick species were found. Nymphs (28%) and larvae (70%) dominated, only one of the 17 individuals had adult ticks (2%). Ticks where almost exclusively found on the axilla and around the neck (photo 3).

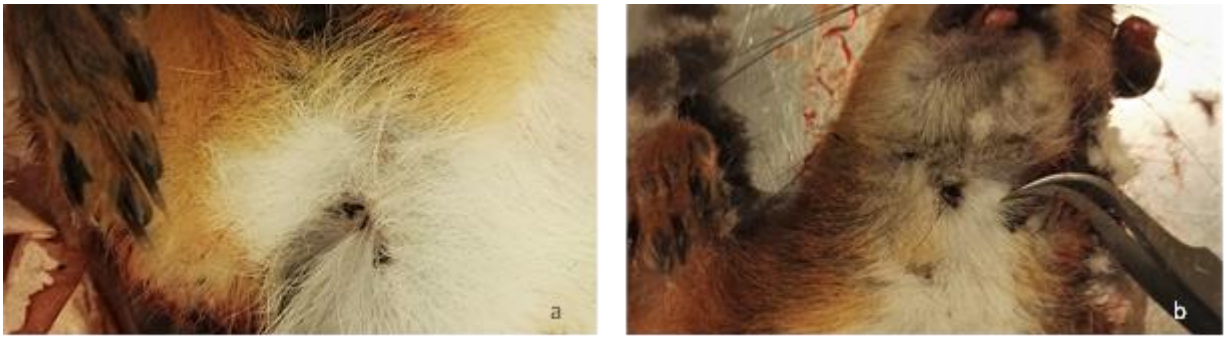


Photo 6a, b: Example of attachment sites on squirrel (photos by Christian Hügli).

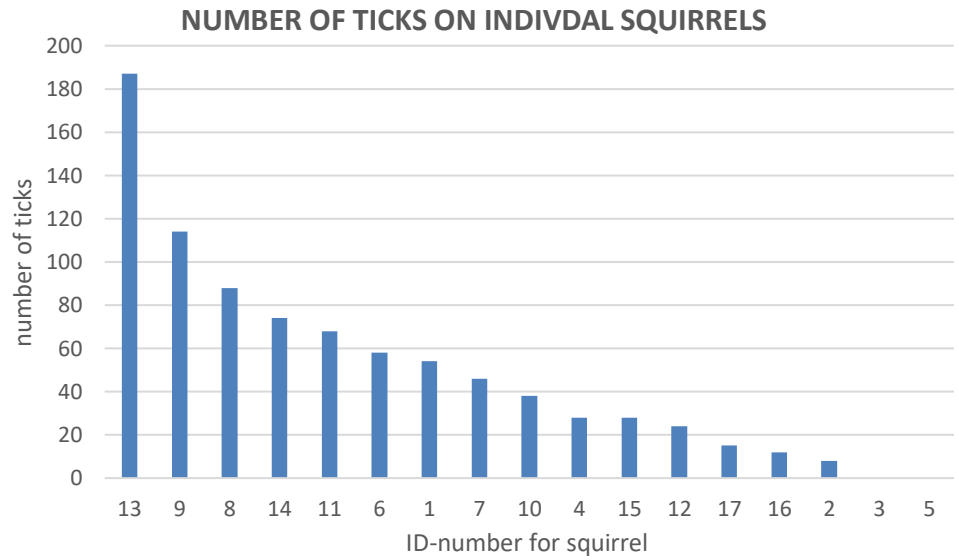


Figure 2: Visualization of number of attached ticks (y-axis) on each individual squirrel (x-axis).

3.2 Comparison of tick load on body parts from different species

The generalized linear mixed effect models of ticks on hosts on specific attachment sites results showed that roe deer had higher tick loads than both red fox and badger, while red fox had higher tick load than badger. Overall, the belly and head are the attachment sites that are most frequently used, followed by the neck and finally the legs.

Table 3. Results of generalized linear mixed effect models of ticks on hosts on specific attachment sites. The baseline for body part was back. Baseline for species was badger.

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-1.525	0.384	-3.970	<0.001
Part:belly	-0.320	0.257	-1.244	0.214
Part:head	0.208	0.257	0.811	0.418
Part:leg	-1.029	0.241	-4.275	<0.001
Part:neck	0.446	0.275	1.621	0.105
Species:red fox	1.742	0.468	3.723	0.001
Species:roe deer	3.526	0.346	10.202	<0.0001

3.3 Presence of pathogens

The presence of pathogens *B. burgdorferi* s.l. and *A. phagocytophilum* varied across the hosts. A high prevalence of *A. phagocytophilum* such as 82 % and the absence of *B. burgdorferi* s.l. were as expected for roe deer (Table 4). A total of 14% of the badgers were infected by *A. phagocytophilum* and also 14% by *B. burgdorferi* s.l.. No coinfection occurred, meaning none of the badgers and red foxes infected with *B. burgdorferi* were also infected by *A. phagocytophilum*. A total of 17% of the red foxes were infected by *A. phagocytophilum*, and also 17% were infected by *B. burgdorferi* s.l. As many as 88% of the squirrels were infected by *B. burgdorferi* s.l..

Table 4. The results from the rtPCR protocol to determine the presence of pathogens *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum.*

Species		*B. burgdorferi* s.l.			*A. phagocytophilum*		
	n	Neg	Pos	Prev	Neg	Pos	Prev
Roe deer	28	28	0	0	5	23	0.82
Badger	14	12	2	0.14	12	2	0.14
Red fox	6	5	1	0.17	5	1	0.17
Squirrel	17	2	15	0.88	17	0	0

4 Discussion

Tick-borne diseases are emerging in northern areas of Europe. Understanding the role of different hosts for limitation of tick-borne diseases is important, as hosts can be managed and provide one potential avenue for mitigation. *I. ricinus* is considered a generalist tick (Mysterud et al. 2019), but host selection of ticks varies across the life stages and can lead to a complicated regulation of tick densities. Deer species are regarded as the dominating host to adult ticks. It has been heavily debated in the literature the extent to which other medium to large sized hosts can serve as reproduction hosts to ticks. It is argued that deer density is not very important if there is access to other large sized hosts (Ostfeld et al. 2006). This is a critical issue in the population regulation of ticks, and hence of considerable management interest (Gilbert et al. 2012). My results shows that the roe deer was the most competent host for in particular adult ticks, as evidenced from both prevalence (%), density and proportion of adult ticks, while squirrels mostly were an important host for nonadult ticks (Table 1). Badgers do not seem to have such a significant role for the ticks lifecycle (Hofmeester, Krawczyk, et al. 2018), while red foxes seemed more important for adult ticks even though not necessarily positively for the parasite (Table 2).

The host competence of medium-sized and large mammals for ticks

The role of a given host for ticks will depend both of its competence to feed different life stages of ticks, and on the relative population density of hosts in the ecosystems. However, how the life stages are attached have only been studied on roe deer in Germany (Kiffner et al. 2010, 2011) and on red deer in Norway (Mysterud, Hatlegjerde, and Sørensen 2014). My study shows a high competence of roe deer as hosts to ticks, in particular adults. Larvae were found exclusively on the feet on the part named leg and, on the head, there especially in the nasal area and on the ears. Nymphs were found mostly on the legs, again more specifically the feet. Adult females were in general in the groin and armpits on the belly parts, but even more on the back and neck. The male ticks were only found on the underside of the female ticks and then clearly more on the neck and back part of the mammals. My study provide further evidence that roe deer are important hosts to adult ticks. This has implications for roe deer management and provide one avenue to regulate tick population in order to fight Lyme disease in humans (more below).

There is only qualitative information on the extent to which badgers and red fox serve as hosts to ticks (Mehl 1983.). Badger are fairly common in urban and suburban habitat spending a lot of time feeding in habitat suitable for ticks. The data presented here nevertheless document that badger are likely to play a minor role for ticks, perhaps due to their thick skin. There is no *I. hexagonus* found on badgers or red foxes. The discovery of subcutaneous ticks in red foxes is interesting. Since the ratio of subcutaneous ticks to external ticks is so high (table 2), it can be assumed that red foxes are not the most competent hosts and that they have some negative impact on the life cycle of the ticks (D'Amico et al. 2017; Haut et al. 2020). In addition, the red fox has a high proportion of small rodents such as mice in its diet which not will benefit the tick population neither (Hofmeester, Jansen, et al. 2018). Hence, the presence of medium-sized mammals like badgers and red fox are not likely to amplify the tick population, in contrast to roe deer.

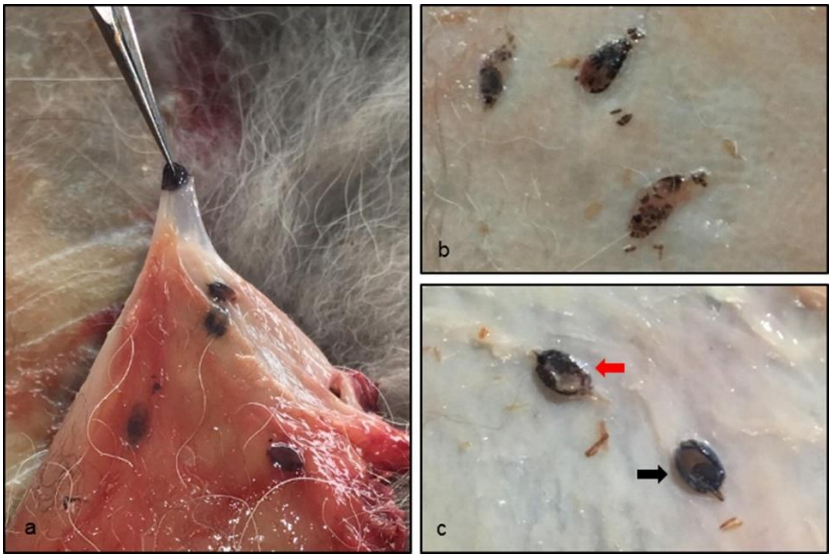


Photo 7 a-c: Macroscopic view on ticks with subcutaneous localization in red fox. (a) One tick lifted up with a fine-tipped forceps and other ticks in the surrounding tissue. (b) Ticks in advanced stages of decomposition. (c) Well-preserved ticks in dorsal (black arrow) and ventral position (red arrow)" (From: Under the skin: *Ixodes* ticks in the subcutaneous tissue of red foxes from Germany).

Although predators are less numerous than herbivores, it would be interesting to have data from, for example, pine marten (*Martes martes*) as well. As a predator of the squirrel, they share the same habitat of the squirrels and the ticks. Because of the size, martens make a suitable host for adult ticks which would let the ticks to fulfil their life cycle (Hofmeester, Krawczyk, et al. 2018).

Squirrels as mentioned, play mostly a role for nonadult ticks. Once tick-infected, there were more than 50 ticks on over 45% of the squirrels in my study and even up to 187 ticks on a single squirrel (Figure 1), which seems to make them really host competent for ticks.

The infection pattern of tick-borne pathogens

Roe deer was a competent host to adult ticks, but they had no infection of *B. burgdorferi* s.l. This is consistent with a number of earlier studies, and it has even been suggested that roe deer clean out *B. burgdorferi* s.l. from the population of ticks (Richter and Matuschka 2006). In contrast, there was high infection of *A. phagocytophilum*, consistent with earlier studies (Mysterud et al. 2017; Razanske et al. 2019; Stigum et al. 2019). The badgers and the red foxes with 14% and 17% infected individuals by both *B. burgdorferi* s.l. and *A. phagocytophilum* seem to play a small or no role for the transmission cycles of these tick-borne pathogens. As mentioned, I did not find any *I. hexagonus*, which is vector-competent for *B. burgdorferi* s.l. Hence, their occurrence would have had an impact to further conclusions for management on large sized hosts (Walker 2018; Mannelli et al. 2012). I did not expect the carnivores to be infected by *A. phagocytophilum*, but there are studies about it from Portugal and Poland (Cardoso et al. 2015; Szewczyk et al. 2019). The fact that the red fox is an effective predator to rodents may also have a negative effect on the spread or density of *B. burgdorferi* s.l. (Hofmeester, Jansen, et al. 2018).

Almost all squirrels in my study (88%) had *B. burgdorferi* s.l. Hence, squirrels are important for the transmission cycles of *B. burgdorferi* s.l.. For squirrels, there are many studies of relationship between *B. burgdorferi* s.l. genospecies, squirrels and *I. ricinus* in European countries (Humair and Gern 1998; Mannelli et al. 2012; Paulauskas et al. 2008). These studies show high prevalence of *B. burgdorferi* s.l. genospecies in different regions (Paulauskas et al. 2008). They point to squirrels as being highly infested with *I. ricinus* being infected with *B. burgdorferi* s.l. (Humair and Gern 1998), but also that in a multi-host system generalist as woodmouse (*Apodemus sylvaticus*), despite annual fluctuations may be more important B. *burgdorferi* s.l. reservoirs (Mannelli et al. 2012). None of the squirrels in my study were infected by *A. phagocytophilum* (Ruyts et al. 2017).

5 Conclusion and management implications

This study confirm that roe deer are particularly competent host to ticks, which can be relevant for the management of roe deer (Mysterud et al. 2016). Since roe deer is a competent host to adult ticks, it may lead to a larger tick population. Hence, decimating the roe deer population might to an extend limit tick abundance but do not have such a big effect to the incidence of *B. burgdorferi* s.l. (Hofmeester et al. 2017). This is because roe deer possibly contribute to a population of ticks that have lower infection prevalence of *B. burgdorferi* s.l. (Mysterud et al. 2013). Several studies showed that ticks cannot pass *B. burgdorferi* s.l. to ruminants such as roe deer, red deer and moose (*Alces alces)* and also farm animals such as goats, sheep and cattle; "These ruminants appear to exert a zooprophylactic effect" (Richter and Matuschka 2010). Further on, infected ticks that suck blood on ruminants lose *B. burgdorferi* s.l. during the blood meal and are no longer infectious when they develop to their next live stage (Richter and Matuschka 2006; Kugeler et al. 2016). So, the potential effect of deer management on decreasing *B. burgdorferi* s.l. remains still controversial and more knowledge about the zooprophylactic importance of the deer species is required.
The high prevalence of *B. burgdorferi* s.l. in the squirrel as a competent host for non-adult ticks indicates that reducing the roe deer, as most important host for adult ticks, population only will have an uncertain effect for the emergence of tick-borne diseases. Even if a smaller roe deer population leads to a smaller tick population, this tick population may then have a higher prevalence of *B. burgdorferi* s.l., be because the squirrel is such an efficient reservoir for the pathogen (Humair and Gern 1998; Paulauskas et al. 2008). The influence of other larger host animals such as badger and red fox seems not to be significant for fulfilling the life cycle of ticks (Hofmeester, Krawczyk, et al. 2018). On the other hand, badger and red fox can "lower the number of ticks feeding on reservoir-competent hosts, which implies that changes in predator abundance may have cascading effects on tick-borne disease risk" (Hofmeester, Jansen, et al. 2018) since they prey on rodents. Many factors play a role and there are many uncertain variables to come to a safe conclusion (Mannelli et al. 2012; Estrada-Peña et al. 2016; Robertson et al. 2019).

6 References

Blindheim, Terje , and Kjell Magne Olsen. 2004. "Kartlegging av naturtyper. Verdisetting av biologisk mangfold i Vestby kommune " In *Siste Sjanse – rapport 2004-8*, 23.

Cardoso, Luís, Matan Gilad, Helder C. E. Cortes, Yaarit Nachum-Biala, Ana Patrícia Lopes, Maria João Vila-Viçosa, Margarida Simões, Paula A. Rodrigues, and Gad Baneth. 2015. 'First report of Anaplasma platys infection in red foxes (Vulpes vulpes) and molecular detection of Ehrlichia canis and Leishmania infantum in foxes from Portugal', *Parasit Vectors*, 8: 144-44.

D'Amico, G., J. Juránková, F. A. Tăbăran, L. Frgelecová, P. Forejtek, I. A. Matei, A. M. Ionică, A. Hodžić, D. Modrý, and A. D. Mihalca. 2017. 'Occurrence of ticks in the subcutaneous tissue of red foxes, Vulpes vulpes in Czech Republic and Romania', *Ticks Tick Borne Dis*, 8: 309-12.

Estrada-Peña, Agustín, Hein Sprong, Alejandro Cabezas-Cruz, José de la Fuente, Ana Ramo, and Elena Claudia Coipan. 2016. 'Nested coevolutionary networks shape the ecological relationships of ticks, hosts, and the Lyme disease bacteria of the Borrelia burgdorferi (s.l.) complex', *Parasit Vectors*, 9: 517-17.

Gilbert, L., G. L. Maffey, S. L. Ramsay, and A. J. Hester. 2012. 'The effect of deer management on the abundance of Ixodes ricinus in Scotland', *Ecol Appl*, 22: 658-67.

Haut, Maja, Nina Król, Anna Obiegala, Johannes Seeger, and Martin Pfeffer. 2020. 'Under the skin: Ixodes ticks in the subcutaneous tissue of red foxes (Vulpes vulpes) from Germany', *Parasit Vectors*, 13: 189-89.

Hofmeester, Tim R., Patrick A. Jansen, Henikus J. Wijnen, Elena C. Coipan, Manoj Fonville, Herbert H. T. Prins, Hein Sprong, and Sipke E. van Wieren. 2018. 'Cascading effects of predator activity on tick-borne disease risk'.

Hofmeester, Tim R., Aleksandra I. Krawczyk, Arieke Docters van Leeuwen, Margriet G. E. Montizaan, Koen van den Berge, Manoj Fonville, Jan Gouwy, Sanne C. Ruyts, Kris Verheyen, and Hein Sprong. 2018. 'Role of mustelids in the life-cycle of ixodid ticks and transmission cycles of four tick-borne pathogens'.

Hofmeester, Tim R., Hein Sprong, Patrick A. Jansen, Herbert H. T. Prins, and Sipke E. van Wieren. 2017. 'Deer presence rather than abundance determines the population density of the sheep tick, Ixodes ricinus, in Dutch forests', *Parasit Vectors*, 10: 433-33.

Humair, Pierre-François, and Lise Gern. 1998. 'Relationship between Borrelia burgdorferi sensu lato species, red squirrels (Sciurus vulgaris) and Ixodes ricinus in enzootic areas in Switzerland', *Acta Trop*, 69: 213-27.

Jones, Kate E., Nikkita G. Patel, Marc A. Levy, Adam Storeygard, Deborah Balk, John L. Gittleman, and Peter Daszak. 2008. 'Global trends in emerging infectious diseases', *Nature*, 451: 990-93.

Jore, Solveig, Hildegunn Viljugrein, Merete Hofshagen, Hege Brun-Hansen, Anja B. Kristoffersen, Karin Nygård, Edgar Brun, Preben Ottesen, Bente K. Sævik, and Bjørnar Ytrehus. 2011. 'Multi-source analysis reveals latitudinal and altitudinal shifts in range of Ixodes ricinus at its northern distribution limit', *Parasit Vectors*, 4: 84-84.

Kiffner, C., C. Lödige, M. Alings, T. Vor, and F. Rühe. 2010. 'Attachment site selection of ticks on roe deer, Capreolus capreolus', *Exp Appl Acarol*, 53: 79-94.

———. 2011. 'Body-mass or sex-biased tick parasitism in roe deer (Capreolus capreolus)? A GAMLSS approach', *Med Vet Entomol*, 25: 39-45.

Kugeler, K. J., R. A. Jordan, T. L. Schulze, K. S. Griffith, and P. S. Mead. 2016. 'Will Culling White-Tailed Deer Prevent Lyme Disease?', *Zoonoses Public Health*, 63: 337-45.

Mannelli, Alessandro, Luigi Bertolotti, Lise Gern, and Jeremy Gray. 2012. 'Ecology of Borrelia burgdorferi sensu lato in Europe: transmission dynamics in multi-host systems, influence of molecular processes and effects of climate change', *FEMS Microbiol Rev*, 36: 837-61.

Mehl, Reidar. 1983. 'The distribution and host relations of Norwegian ticks (Acari, Ixodides)', *Fauna norvegica* Series B 30 NO.1: 46-51.

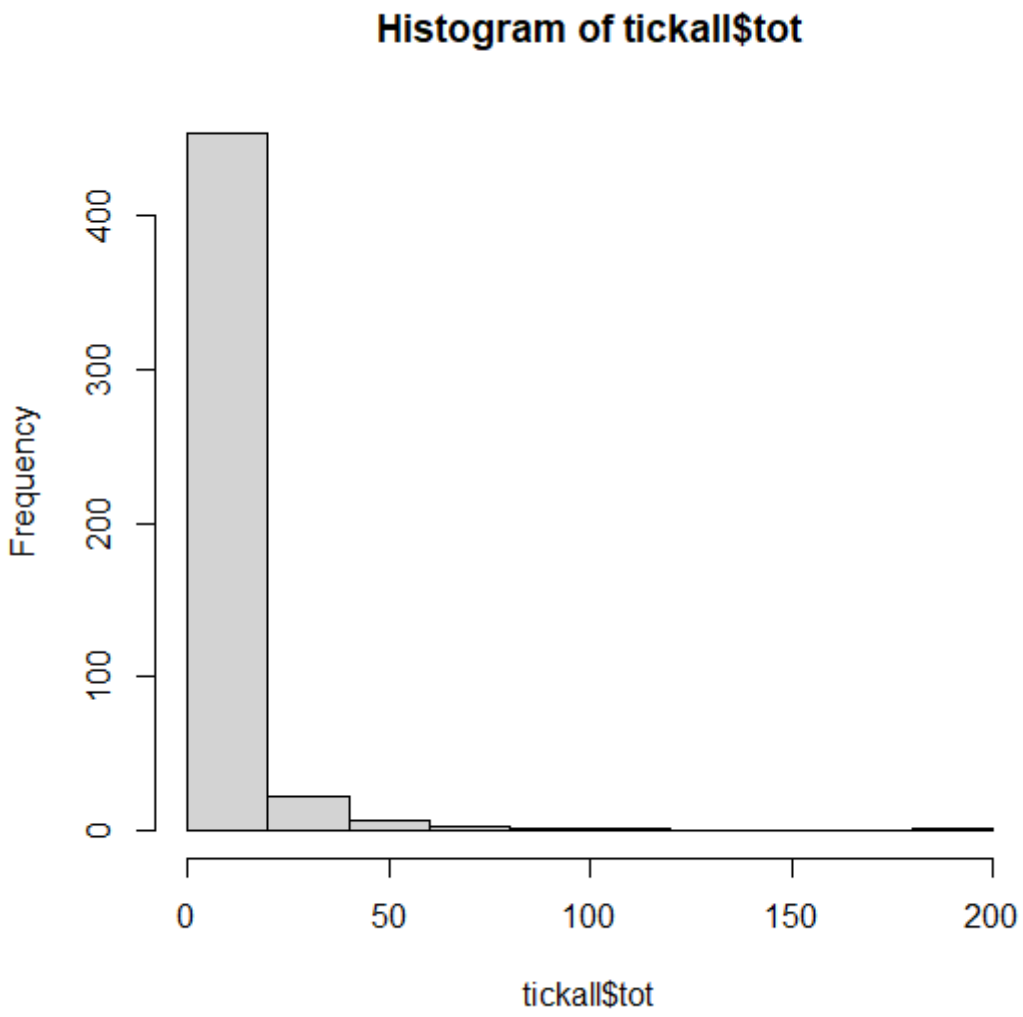
Moen, Asbjørn. 1998. *Vegetasjon* (Norges geografiske oppmåling: [Hønefoss]).
Mysterud, Atle, William Ryan Easterday, Lars Qviller, Hildegunn Viljugrein, and Bjørnar Ytrehus. 2013. 'Spatial and seasonal variation in the prevalence of Anaplasma phagocytophilum and Borrelia burgdorferi sensu lato in questing Ixodes ricinus ticks in Norway', *Parasit Vectors*, 6: 187-87.
Mysterud, Atle, William Ryan Easterday, Vetle Malmer Stigum, Anders Bjørnsgard Aas, Erling L. Meisingset, and Hildegunn Viljugrein. 2016. 'Contrasting emergence of Lyme disease across ecosystems'.
Mysterud, Atle, Idar Lauge Hatlegjerde, and Ole Jakob Sørensen. 2014. 'Attachment site selection of life stages of Ixodes ricinus ticks on a main large host in Europe, the red deer (Cervus elaphus)', *Parasit Vectors*, 7: 510.
Mysterud, Atle, Solveig Jore, Olav ster?s, and Hildegunn Viljugrein. 2017. 'Emergence of tick-borne diseases at northern latitudes in Europe: a comparative approach', *Sci Rep*, 7: 16316-12.
Mysterud, Atle, Vetle Malmer Stigum, Harald Linløkken, Anders Herland, and Hildegunn Viljugrein. 2019. 'How general are generalist parasites? The small mammal part of the Lyme disease transmission cycle in two ecosystems in northern Europe', *Oecologia*, 190: 115-26.
Ostfeld, Richard S., Charles D. Canham, Kelly Oggenfuss, Raymond J. Winchcombe, and Felicia Keesing. 2006. 'Climate, Deer, Rodents, and Acorns as Determinants of Variation in Lyme-Disease Risk', *PLoS biology*, 4: e145.
Parmesan, Camille. 2006. 'Ecological and Evolutionary Responses to Recent Climate Change', *Annual review of ecology, evolution, and systematics*, 37: 637-69.
Paulauskas, Algimantas, Daiva Ambrasiene, Jana Radzijevskaja, Olav Rosef, and Jurga Turcinaviciene. 2008. 'Diversity in prevalence and genospecies of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and rodents in Lithuania and Norway', *International Journal of Medical Microbiology*, 298: 180-87.
Razanske, Irma, Olav Rosef, Jana Radzijevskaja, Maksim Bratchikov, Loreta Griciuviene, and Algimantas Paulauskas. 2019. 'Prevalence and co-infection with tick-borne Anaplasma phagocytophilum and Babesia spp. in red deer (Cervus elaphus) and roe deer (Capreolus capreolus) in Southern Norway', *Int J Parasitol Parasites Wildl*, 8: 127-34.
Richter, Dania, and Franz-Rainer Matuschka. 2006. 'Modulatory effect of cattle on risk for lyme disease', *Emerg Infect Dis*, 12: 1919-23.
Richter, Dania, and Franz-Rainer Matuschka. 2010. 'Elimination of Lyme Disease Spirochetes from Ticks Feeding on Domestic Ruminants', *Appl Environ Microbiol*, 76: 7650-52.
Robertson, Daniel L., Leah M. Babin, Jenna R. Krall, Michael E. von Fricken, Heibatollah Baghi, and Kathryn H. Jacobsen. 2019. 'The Association Between Hunter-Killed Deer and Lyme Disease in New Jersey, 2000–2014', *Ecohealth*, 16: 330-37.
Ruyts, Sanne C., Ewa Frazer-Mendelewska, Koen Van Den Berge, Kris Verheyen, and Hein Sprong. 2017. 'Molecular detection of tick-borne pathogens Borrelia afzelii, Borrelia miyamotoi and Anaplasma phagocytophilum in Eurasian red squirrels (Sciurus vulgaris)', *European journal of wildlife research*, 63: 1.
Sprong, Hein, Tal Azagi, Dieuwertje Hoornstra, Ard M. Nijhof, Sarah Knorr, M. Ewoud Baarsma, and Joppe W. Hovius. 2018. 'Control of Lyme borreliosis and other Ixodes ricinus-borne diseases', *Parasit Vectors*, 11: 145-45.
Stigum, Vetle M., Ryanne I. Jaarsma, Hein Sprong, Christer M. Rolandsen, and Atle Mysterud. 2019. 'Infection prevalence and ecotypes of Anaplasma phagocytophilum in moose Alces alces, red deer Cervus elaphus, roe deer Capreolus capreolus and Ixodes ricinus ticks from Norway', *Parasit Vectors*, 12: 1-1.
Szewczyk, Tomasz, Joanna Werszko, Anna W. Myczka, Zdzisław Laskowski, and Grzegorz Karbowiak. 2019. 'Molecular detection of Anaplasma phagocytophilum in wild carnivores in north-eastern Poland', *Parasit Vectors*, 12: 465-65.
Walker, Mark David. 2018. 'The hedgehog tick, Ixodes hexagonus (Leach, 1815) (Acari: Ixodidae); The natural history and ecology of a nest ectoparasite', *Systematic and applied acarology*, 23: 680.

Appendix

Table 1. Overview about parts with ticks on different hosts with tick prevalence, intensity, proportion of life stages and overall density (ticks per cm^2).

species		n	Prevalence (%)	Intensity (ticks per part)	intensity (larvae) per part)	intensity (nymphs per part)	intensity (adult males per part)	intensity (adults females per part)	intensity (adults per part)	Prop larvae	Prop. Nymphs	Prop adult	density
Roe deer	Roe deer	29	100.00	8.85	0.22	2.35	1.57	4.70	6.27	0.03	0.27	0.71	0.028
part	back	29	100.00	12.53	0.00	0.21	4.00	8.32	12.32	0.00	0.02	0.98	0.064
	belly b	29	100.00	7.93	0.07	0.81	1.15	5.89	7.04	0.01	0.10	0.89	0.036
	belly f	29	100.00	5.91	0.09	0.59	1.09	4.14	5.23	0.02	0.10	0.88	0.026
	ear	29	100.00	8.83	0.00	7.50	0.58	0.75	1.33	0.00	0.85	0.15	NA
	head	29	100.00	10.95	0.05	1.33	1.57	8.00	9.57	0.00	0.12	0.87	NA
	leg b	29	100.00	6.67	1.33	5.00	0.27	0.07	0.33	0.20	0.75	0.05	0.058
	leg f	29	100.00	6.50	0.86	5.21	0.14	0.29	0.43	0.13	0.80	0.07	0.076
	neck	29	100.00	15.36	0.00	0.18	4.36	10.82	15.18	0.00	0.01	0.99	0.052
	shoulder	29	100.00	6.40	0.40	2.20	0.93	2.87	3.80	0.06	0.34	0.59	0.023
	thigh	29	100.00	5.00	0.06	1.71	0.76	2.47	3.24	0.01	0.34	0.65	0.018
part2	back	29	100.00	12.53	0.00	0.21	4.00	8.32	12.32	0.00	0.02	0.98	0.064
	belly	58	100.00	7.02	0.08	0.71	1.12	5.10	6.22	0.01	0.10	0.89	0.031
	head	58	100.00	9.82	0.02	4.62	1.04	4.13	5.18	0.00	0.47	0.53	NA
	leg	116	100.00	6.10	0.64	3.44	0.54	1.48	2.02	0.10	0.56	0.33	0.042
	neck	29	100.00	15.36	0.00	0.18	4.36	10.82	15.18	0.00	0.01	0.99	0.052
Badger	Badger	14	71.43	1.40	0.00	0.07	0.07	1.27	1.33	0.00	0.05	0.95	0.001
part	back	14	7.14	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.016
	belly l.	14	14.29	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.005
	belly r.	14	21.43	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.007
	head	14	7.14	3.00	0.00	1.00	0.00	2.00	2.00	0.00	0.33	0.67	NA
	leg b.l.	14	7.14	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.016
	leg b.r.	14	14.29	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.012
	leg f.l.	14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA	NA	0.000
	leg f.r.	14	7.14	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.008
	neck	14	21.43	2.00	0.00	0.00	0.25	1.75	2.00	0.00	0.00	1.00	0.014
part2	back	14	7.14	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.016
	belly	28	17.86	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.006
	head	14	7.14	3.00	0.00	1.00	0.00	2.00	2.00	0.00	0.33	0.67	NA
	leg	56	7.14	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.012
	neck	14	21.43	2.00	0.00	0.00	0.25	1.75	2.00	0.00	0.00	1.00	0.014

Fox	Fox	6	100.00	2.94	0.00	0.00	0.24	2.71	2.94	0.00	0.00	1.00	0.002
part	back	6	33.33	2.50	0.00	0.00	0.00	2.50	2.50	0.00	0.00	1.00	0.008
	belly l.	6	33.33	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.012
	belly r.	6	16.67	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.007
	head	6	83.33	5.80	0.00	0.00	0.60	5.20	5.80	0.00	0.00	1.00	NA
	leg b.l.	6	16.67	2.00	0.00	0.00	0.00	2.00	2.00	0.00	0.00	1.00	0.007
	leg b.r.	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA	NA	0.000
	leg f.l.	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA	NA	0.000
	leg f.r.	6	33.33	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.004
	neck	6	66.67	2.25	0.00	0.00	0.25	2.00	2.25	0.00	0.00	1.00	0.014
part2	back	6	33.33	2.50	0.00	0.00	0.00	2.50	2.50	0.00	0.00	1.00	0.008
	belly	12	25.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	1.00	0.010
	head	6	33.33	5.80	0.00	0.00	0.60	5.20	5.80	0.00	0.00	1.00	NA
	leg	24	12.50	1.33	0.00	0.00	0.00	1.33	1.33	0.00	0.00	1.00	0.005
	neck	6	33.33	2.25	0.00	0.00	0.25	2.00	2.25	0.00	0.00	1.00	0.014
Squirrel	Squirrel	17	88.24	56.13	15.73	39.40	0.33	0.67	1.00	0.28	0.70	0.02	2.014



Figur: Negativ binominal distribution

Family: nbinom2 (log)
Formula: tot ~ part2 + species1 + (1 | ID1)
Zero inflation: ~1
Data: tickall

AIC	BIC	logLik	deviance	df.resid
1783.2	1824.7	-881.6	1763.2	460

Random effects:

Conditional model:

Groups	Name	Variance	Std.Dev.
ID1	(Intercept)	0.4062	0.6373

Number of obs: 470, groups: ID1, 49

Overdispersion parameter for nbinom2 family (): 1.17

Conditional model:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-1.525	0.384	-3.970	<0.001	***
part2belly	-0.320	0.257	-1.244	0.214	
part2head	0.208	0.257	0.811	0.418	
part2leg	-1.029	0.241	-4.275	<0.001	***
part2neck	0.446	0.275	1.621	0.105	
species1fox	1.742	0.468	3.723	0.001	***
species1roe_deer	3.526	0.346	10.202	<0.0001	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Zero-inflation model:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-1.6729	0.3868	-4.325	1.53e-05	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	
(Intercept)	-1.525	0.384	-3.970	<0.001	***
part2belly	-0.320	0.257	-1.244	0.214	
part2head	0.208	0.257	0.811	0.418	
part2leg	-1.029	0.241	-4.275	<0.001	***
part2neck	0.446	0.275	1.621	0.105	
species1fox	1.742	0.468	3.723	0.001	***
species1roe_deer	3.526	0.346	10.202	<0.0001	***

ID	day	month	year	location	species	age	sex	weight	lenght	part	part2	areal	larvae	nymphs	adult mal	adult fem	tot	tickornot	density
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	leg b.	leg	99	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	tight	leg	204	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	belly b.	belly	185	0	0	3	24	27	yes	0.145946
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	belly f.	belly	172	0	0	0	1	1	yes	0.005814
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	leg f.	leg	50	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	shoulder	leg	133	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	neck	neck	218	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	back	back	234	0	0	1	1	2	yes	0.008547
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	ear	head	NA	0	11	0	0	11	yes	NA
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	head	head	NA	0	0	0	0	0	no	NA

ID	day	month	year	location	species	age	sex	weight	lenght	part	part2	areal	larvae	nymphs	adult male	adult female	tot	tickornot	density
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	leg b.	leg	99	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	tight	leg	204	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	belly b.	belly	185	0	0	3	24	27	yes	0.145946
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	belly f.	belly	172	0	0	0	1	1	yes	0.005814
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	leg f.	leg	50	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	shoulder	leg	133	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	neck	neck	218	0	0	0	0	0	no	0
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	back	back	234	0	0	1	1	2	yes	0.008547
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	ear	head	NA	0	11	0	0	11	yes	NA
RA001	NA	may	2015	Droebak	roe_deer	yearling	female	NA	70	head	head	NA	0	0	0	0	0	no	NA

Table.

Species		*B. burgdorferi* sl			*A. phagocytophilum*		
	n	Neg	Pos	Prev	Neg	Pos	Prev
Roe deer	28	28	0	0	5	23	0.82
Badger	14	12	2	0.14	12	2	0.14
Red fox	6	5	1	0.17	5	1	0.17
Red squirrel	17	2	15	0.88	17	0	0

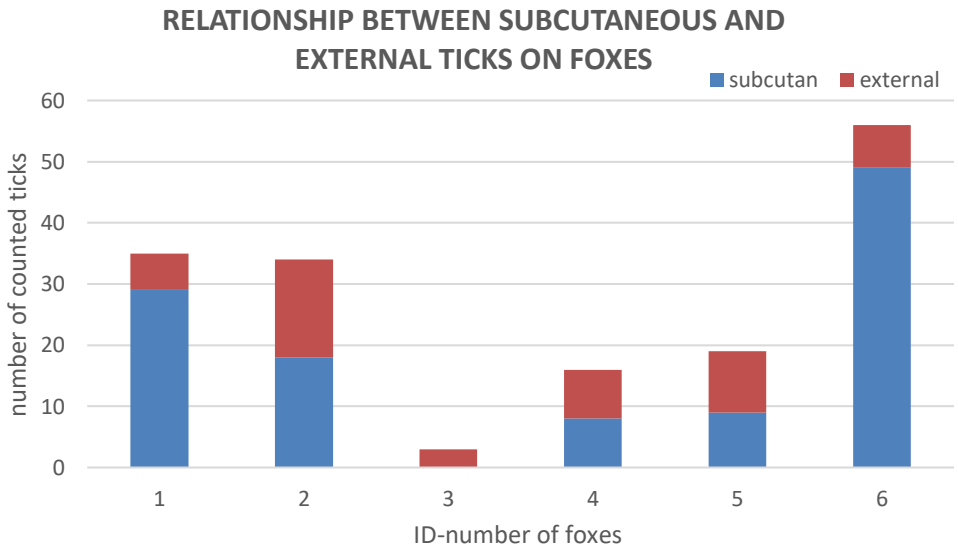


Figure 1: Visualization of relationship between subcutaneous and external ticks on foxes. The y-axis shows the total number of ticks found. On the x-axis are the individual foxes.

Subcutaneous ticks

	RE001	RE002	RE003	RE004	RE005	RE006
back	0	0	0	0	0	0
belly	3	5	0	0	0	8
head	NA	NA	NA	NA	NA	NA
legg	26	13	0	8	9	41
neck	0	0	0	0	0	0

External Ticks

	RE001	RE002	RE003	RE004	RE005	RE006
back	1	4	0	0	0	0
belly	0	0	1	1	1	0
head	1	6	0	7	8	7
legg	3	1	0	0	0	0
neck	1	5	2	0	1	0

TickDeer

DNA Extraction **Plate: 89**

Date: 15.2.17

	1	2	3	4	5	6	7	8	9
A	RA001	RA009	RA017	RA025	GR005	GR013	HA001	EK008	EK016
B	RA002	RA010	RA018	RA026	GR006	GR014	EK001	EK009	EK017
C	RA003	RA011	RA019	RA027	GR007	RE001	EK002	EK010	
D	RA004	RA012	RA020	RA028	GR008	RE002	EK003	EK011	
E	RA005	RA013	RA021	GR001	GR009	RE003	EK004	EK012	
F	RA006	RA014	RA022	GR002	GR010	RE004	EK005	EK013	
G	RA007	RA015	RA023	GR003	GR011	RE005	EK006	EK014	
H	RA008	RA016	RA024	GR004	GR012	RE006	EK007	EK015