Ticking off the ungulate box

The role of different ungulate species in the transmission of tick-borne pathogens

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Abstract

Ungulates play a central role in the life cycle of *Ixodes ricinus*, an important vector of tick-borne pathogens, and several ungulate species are increasingly common across Europe. I investigated the role of these different species in the spread of *I. ricinus*-borne pathogens. Through a meta-analysis, I quantified the relative importance of ungulate species in the transmission of *Anaplasma phagocytophilum*. Furthermore, through field studies, I compared the contribution of each species to the number of ticks and the transmission of *A. phagocytophilum* and *Borrelia burgdorferi* sensu lato (s.l.) by quantifying tick burdens, relative ungulate densities, vegetation structure and (infected) questing tick density. My studies indicated that deer contributed more to the spread of tick-borne pathogens than wild boar (*Sus scrofa*), and fallow deer (*Dama dama*) more than the other deer species. I then modelled how changing an ungulate community composition affects the establishment of pathogens, expressed by the reproduction number R_0. High density of fallow deer along with low density of roe deer (*Capreolus capreolus*) resulted in a higher R_0 of the zoonotic *A. phagocytophilum* ecotype 1, and a lower R_0 of the non-zoonotic ecotype 2. The effects of ungulates on the R_0 of *B. afzelii* and *B. garinii* were negligible. My thesis thus suggests that different deer species likely vary in their effect on the circulation of various tick-borne pathogens. Ungulate management, as a tool to mitigate public and veterinary health risk, should therefore not approach ungulates as one homogenous group, but depending on the pathogen, take note of potentially different roles that ungulate species may play.

Keywords: *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, emerging infectious disease, *Ixodes ricinus*, One Health, tick-borne pathogen, transmission, ungulate management, zoonosis

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Ticking off the ungulate box – rollen av olika klövviltarters i överföringen av fästingburna patogener

Sammanfattning

Flera klövviltarter blir allt vanligare i Europa. Just klövvilt spelar en central roll i livscykeln för fästingen *Ixodes ricinus*, som är en viktig vektor för fästingburna patogener. Jag har undersökt vilken roll de olika klövviltsarterna har för spridningen av *I. ricinus*-burna patogener. Genom en metaanalys kvantifierade jag den relativa betydelsen av klövviltarter för spridning av *Anaplasma phagocytophilum*. Dessutom har jag genom fältstudier jämfört de enskilda klövviltsarternas bidrag till spridningen av *A. phagocytophilum* och *Borrelia burgdorferi* sensu lato (s.l.) genom att kvantifiera antalet fästingar på klövvilt, relativa klövviltstätheter, vegetationsstruktur och tätheter av värdsökande fästingar. Mina studier indikerade att hjortdjuren, framför allt dovhjort (*Dama dama*), bidrog mer till spridningen av fästingburna patogener jämfört med vildsvin (*Sus scrofa*). Jag modellerade sedan hur förändringar i artsammansättningen hos klövvilt påverkar etableringen av patogener, utryckt som reproduktionstalet R_0. Hög täthet av dovhjort tillsammans med låg täthet av rådjur (*Capreolus capreolus*) resulterade i ett högre R_0 för den zoonotiska *A. phagocytophilum* ekotyp 1, och ett lägre R_0 för den icke-zoonotiska ekotypen 2. Effekterna av klövvilt på R_0 av *B. afzelii* och *B. garinii* var försumbara. Min avhandling antyder alltså att olika klövviltsarter sannolikt varierar i sin effekt på cirkulationen av olika fästingburna patogener. Förvaltningen av klövvilt, som ett verktyg för att minska risker för folk- och djurhälsan, bör därför inte hantera klövvilt som en homogen grupp utan – beroende på patogenen – ta hänsyn till de potentiellt olika roller som klövviltsarterna kan ha.

Nyckelord: *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, nya smittsamma sjukdomar, *Ixodes ricinus*, One Health, fästingburen patogen, spridning, förvaltning av klövvilt, zoonos

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Ticking off the ungulate box - de rol van verschillende hoefdieren in de transmissie van teek-overdraagbare pathogenen

Samenvatting

Hoefdieren spelen een centrale rol in de levenscyclus van *Ixodes ricinus*, een belangrijke vector van teek-overdraagbare pathogenen. Verschillende hoefdiersoorten komen steeds vaker in grotere aantallen, en op meerdere plekken, voor in Europa. Ik onderzocht de rol van deze soorten in de verspreiding van door *I. ricinus* overgedragen pathogenen. Door middel van een meta-analyse heb ik het relatieve belang van hoefdiersoorten in de transmissie van *Anaplasma phagocytophilum* gekwantificeerd. Verder heb ik door middel van veldstudies de bijdrage van elke soort aan het aantal teken en de transmissie van *A. phagocytophilum* en *Borrelia burgdorferi* sensu lato (s.l.) vergeleken, door het aantal teken op de hoefdieren, de relatieve dichtheden van hoefdieren, de structuur van de vegetatie en de dichtheden van de zoekende teken te kwantificeren. Deze studies gaven aan dat hertensoorten meer bijdroegen aan de verspreiding van teek-overdraagbare pathogenen dan wilde zwijnen (*Sus scrofa*), en damherten (*Dama dama*) meer dan de andere hertensoorten. Vervolgens heb ik gemodelleerd hoe de samenstelling van de hoefdiersoortengemeenschap de verspreiding van pathogenen beïnvloedt, uitgedrukt in het reproductiegetal R_0. Een hoge dichtheid van damherten, samen met een lage dichtheid van reeën (*Capreolus capreolus*), resulteerde in een hogere R_0 voor het zoönotische *A. phagocytophilum* ecotype 1 en een lagere R_0 voor het non-zoönotische ecotype 2. Het effect van hoefdieren op de R_0 van *B. afzelii* en *B. garinii* was verwaarloosbaar. Mijn proefschrift suggereert dus dat, afhankelijk van het pathogeen, hertensoorten verschillen in hun effect op de verspreiding van teek-overdraagbare pathogenen. In het beheer van hoefdieren, als een middel om de risico's voor de volksgezondheid te verminderen, moeten de hoefdieren daarom niet als één homogene groep worden beschouwd, maar moet er rekening worden gehouden met de verschillende rollen die de verschillende hoefdieren spelen

Dedication

Voor opa Leo –

Jouw kennis en enthousiasme heeft me van jongs af aan gedreven om altijd meer te willen weten. Ik had je er graag bij gehad tot het einde, maar je bent altijd bij me in mijn hart.

"Ik vind het beschamend om de kennis der natuur te verwaarlozen. […] Ik houd er niet van dingen half te weten."

"I find it shameful to neglect the knowledge of nature. […] I do not like it to know things half-heartedly."

Belle van Zuylen
(1740 – 1805)

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Fabri, N.D.*, Sprong, H., Heesterbeek, J.A.P., Ecke, F., Cromsigt, J.P.G.M. & Hofmeester, T.R. (in review) The circulation of *Anaplasma phagocytophilum* ecotypes is associated with community composition of vertebrate hosts.

II. Fabri, N.D.*, Sprong, H., Hofmeester, T.R., Heesterbeek, J.A.P., Donnars, B.F., Widemo, F., Ecke, F. & Cromsigt, J.P.G.M. (2021) Wild ungulate species differ in their contribution to the transmission of *Ixodes ricinus*-borne pathogens. *Parasites & Vectors*. 14:360

III. Fabri, N.D.*, Hofmeester, T.R., Ecke, F., Sprong, H., Timmermans, J., Heesterbeek, J.A.P. & Cromsigt, J.P.G.M. Associations between abundances of different ungulate species, height of the field layer and presence of *Ixodes ricinus* ticks. (manuscript)

IV. Fabri, N.D.*, Heesterbeek, J.A.P., Cromsigt, J.P.G.M., Ecke, F., Sprong, H., Nijhuis, L., Hofmeester, T.R. & Hartemink, N. Composition of host community influences the basic reproduction number of different tick-borne pathogens with possible implications for ungulate management. (manuscript)

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The contribution of Nannet D. Fabri to the papers included in this thesis was as follows:

I. Main author. Designed the study together with HS and TH. Collected data with contributions from all co-authors. Carried out all analyses and visualization with advice from TH. Wrote the manuscript with contributions from the co-authors.

II. Main author. Designed the study together with JC, HS and FW. Collected data in the field with BD. Performed lab analyses with BD and HS. Carried out all analyses and visualization with advice from TH. Wrote the paper with contributions from the co-authors.

III. Main author. Designed the study together with TH, FE, JC and JT. Collected data with JT. Performed lab analyses with HS. Carried out all analyses and visualization with advice from FE, TH and JC. Wrote the manuscript with contributions from the co-authors.

IV. Main author. Designed the study together with JC, JH and NH. Collected data with LN and NH. Carried out all analyses and visualization with advice from JH and NH. Wrote the manuscript with contributions from the co-authors.

Abbreviations and terms

Basic reproduction number (R_0)	The average number of secondary cases caused by the placement of one infectious individual in a population consisting entirely of susceptible individuals (Hartemink et al., 2008).
Field layer vegetation	Vegetation with a height of max. 50cm, including grasses, forbs, dwarf shrubs and mosses.
Infection intensity	The total number of infected ticks on an average individual of a certain host species (Adapted from Kahl et al., 2002).
Infection prevalence	The proportion of examined ticks or hosts infected with a pathogen (Kahl et al., 2002).
Infestation intensity	The average number of ticks on an infested host (Adapted from Kahl et al., 2002).
Infestation prevalence	The proportion of examined hosts that are infested with ticks (Kahl et al., 2002).
Propagation hosts	Hosts that are needed to ensure the reproduction of ticks.
Relative importance	The proportional contribution of a host species to, for example, feeding ticks or transmission, in a certain area in relation to other hosts (Extension of the definition for relative reservoir capacity by Kahl et al., 2002).
Reservoir host	Vertebrates that are capable of transmitting a pathogen.

Tick burden	The total number of ticks on an average individual of a certain host species (Adapted from Kahl et al., 2002).
Vector-borne pathogen	Pathogens transmitted by the bite of an infected arthropod, such as mosquitoes, ticks or flies.
qPCR	Quantitative or real-time polymerase chain reaction.
Questing	The act of seeking a vertebrate host by climbing on field layer vegetation. When a potential host passes the ticks will grab on the animal and seek a site for their blood meal (Ostfeld, 2011).
Zoonotic pathogen	A pathogen that can be transmitted between humans and non-human animals.

1. Introduction

It is the year 2022 and, for the last two years, the world has been under the spell of a new emerging infectious disease: COVID-19, caused by the zoonotic pathogen SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). This virus does not only infect humans, but has also been found in many non-human mammals, including mustelids, cats, dogs and ungulates (Fischhoff et al., 2021), and thus affects the health of humans, domestic animals and wildlife. SARS-CoV-2 is only one example of a growing list of emerging pathogens that may affect the health of a broad range of animals, including humans (Berger, 2005). A subgroup of these pathogens are vector-borne, which means that arthropod vectors, such as ticks and mosquitoes, transport the pathogen between animal hosts. Vector-borne and zoonotic pathogens have been around for a long time and cause well-known human diseases, such as Ebola and Lyme borreliosis, that pose significant burden to society (Huber et al., 2018; van den Wijngaard et al., 2017). Vector-borne and zoonotic diseases are responsible for more than 80% of all emerging infectious diseases (Jones et al., 2008). Parasites of the order Ixodidae, i.e., ticks, play a major role as vector in Europe. One of the most common tick-borne diseases in Europe, Lyme borreliosis, is responsible for the largest disease burden of all vector-borne diseases in the European Union (Semenza & Suk, 2018).

1.1 Ticks and their hosts

Ticks have been present on earth for a long time. A now extinct tick species was found in 99 million-year-old fossilized amber (Peñalver et al., 2017). Also, the Greek philosopher Aristotle mentioned ticks in his *Historia Animalium*, written in 355 B.C. (Arthur, 1965). He wrote: ‘The ass has no

lice or ticks, oxen have both […] among dogs Cynorhaestes are plentiful', where he is believed to refer to the tick species *Ixodes ricinus* with the word Cynorhaestes (Arthur, 1965). Nowadays, there are around 900 known tick species worldwide, divided into two families: *Ixodidae* (hard ticks) and *Argasidae* (soft ticks) (Parola & Raoult, 2001). Specific tick species are usually associated with certain areas of the world. For example, the tick species *I. ricinus* is the most widespread species in Europe (ECDC, 2021) while the blacklegged tick *Ixodes scapularis* is among the most widely distributed ticks in the eastern United States of America (CDC, 2021). Both *I. ricinus* and *I. scapularis* are considered to be generalized parasites (Piesman & Gern, 2004), which means that they can feed on a multitude of host species. However, vertebrate host species differ in their contribution as hosts for these tick species. Ticks from the genus *Ixodes* have four life stages, of which three need a bloodmeal for survival. Previous studies have shown that larvae are mainly found on smaller vertebrates, nymphs feed mainly on small and medium-sized vertebrates, while adults feed mainly on larger vertebrates, such as roe deer (*Capreolus capreolus*) in Europe and white-tailed deer (*Odocoileus virginianus*) in the United States of America (Kollars et al., 1999; Pfäffle et al., 2013; See also Chapter 2).

1.2 Tick-borne pathogens

Many tick species are associated with tick-borne pathogens. Some species, such as *I. scapularis* and *I. ricinus*, can harbour numerous pathogens, while other species are currently associated with one major pathogen (Rochlin & Toledo, 2020). New pathogens, however, are constantly detected (Tokarz & Lipkin, 2021). Some pathogens, usually rare ones, are associated with only one tick species, while others can be found in multiple tick species and consequently in multiple areas of the world (Rochlin & Toledo, 2020). Two of these latter, widespread, pathogens are *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato (s.l.), both transmitted by *I. ricinus*, *I. scapularis* and *Ixodes pacificus*, among others, and occurring in North America, Europe and Northern Asia (Rochlin & Toledo, 2020).

1.2.1 *Anaplasma phagocytophilum*

A. phagocytophilum is a pathogen that can cause granulocytic anaplasmosis in humans and anaplasmosis, or tick-borne fever, in domestic ruminants

(Stuen et al., 2013). It was previously known as a combination of *Ehrlichia phagocytophilia*, *Ehrlichia equi* and *Ehrlichia* 'HE agent' (Dumler et al., 2001). The pathogen has been isolated from a number of wild host species, including roe deer, white-tailed deer, long-tailed field mouse (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*) and white-footed mouse (*Peromyscus leucopus*) (Stuen et al., 2013). Jahfari et al. (2014) proposed four distinct ecotypes of *A. phagocytophilum*, based on genetic differences in the *groEL* gene. These ecotypes seem to be associated with different host groups (Bown et al., 2009; Jaarsma et al., 2019; Jahfari et al., 2014). Ecotype 1 has been found in a wide range of host species, including humans, but the other ecotypes seem to be associated with a specific host group: ecotype 2 with roe deer, ecotype 3 with small mammals and ecotype 4 with birds (Jahfari et al., 2014). Therefore, ecotype 1 is the most important ecotype for human and veterinary health.

1.2.2 *Borrelia burgdorferi* sensu lato

Borrelia burgdorferi s.l. is a collective name for a complex of genospecies that includes 18 named spirochete species (Rudenko et al., 2011). At least three of these species are known to commonly infect humans and cause Lyme borreliosis (or Lyme disease), namely, *Borrelia afzelii*, *Borrelia garinii* and *Borrelia burgdorferi* sensu stricto (s.s.) (Rudenko et al., 2011). The different genospecies have different clinical manifestations of Lyme borreliosis. *B. afzelii*, for example, seems to be associated with skin lesions (e.g., erythema migrans and acrodermatitis chronica atrophicans), while the more severe neuroborreliosis is more associated with *B. garinii* (Coipan et al., 2016). This indicates that the importance for public health varies with genospecies. The different genospecies of the *B. burgdorferi* s.l. complex do not only differ in their clinical manifestations, but also in terms of their main reservoir host. Small mammals, for example, are associated with *B. afzelii*, while birds are associated with *B. garinii* (Hanincova et al., 2003; Taragel'ová et al., 2008). Ungulate species are considered as dead-end hosts for the genospecies of the *B. burgdorferi* s.l. complex (Mannelli et al., 2012; Ostfeld, 2011).

1.3 The rise of *Ixodes ricinus* in Europe

Despite *I. ricinus* already being the most common tick species in Europe, a further expansion of the distribution range and increase in abundance of *I.*

ricinus has been observed over the last few decades (Jaenson et al., 2012; Jore et al., 2011; Sprong et al., 2012). This increase can be attributed to three factors that are not mutually exclusive (Reviewed in Medlock et al., 2013). Firstly, climate change contributes to the expansion of the geographic range of *I. ricinus* towards higher latitudes and altitudes. Secondly, anthropogenic factors, such as changes in land use type and intensity, could facilitate the abundance of *I. ricinus*. Thirdly, changes in the distribution and community composition of tick hosts (which are partly connected to climate change), for example small mammals and ungulates, directly affect tick numbers. The increase in distribution and abundance of *I. ricinus* goes hand in hand with an increase in the prevalence of tick-borne diseases in humans and livestock (Reviewed in Madison-Antenucci et al., 2020), posing a threat to human and veterinary health. However, not only the increase in distribution and abundance of *I. ricinus* plays a role in the increase in prevalence of tick-borne diseases. Changes in the composition of the host community play a major role as well. When ticks cannot find any suitable host for a certain pathogen, the transmission cycle of that pathogen cannot be maintained. Changes in the distribution and composition of host communities can therefore contribute to increased prevalence of tick-borne diseases (Ostfeld & Keesing, 2012).

1.4 Ungulates and ticks

Ungulates are one of the main hosts for *I. ricinus* (Hofmeester et al., 2016), and their numbers have been strongly increasing across Europe in the last 4-5 decades (Apollonio et al., 2010; Spitzer, 2019). In addition to increasing numbers, many ungulate species, such as roe deer, wild boar (*Sus scrofa*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus*), have recolonized large parts of their historic range, after being extirpated across large parts of Europe by the early 20th century (Deinet et al., 2013). Moreover, several of these species are expanding northwards, possibly due to warmer winters, into areas where they did not historically occur (Apollonio et al., 2010). Large parts of Europe, and especially Scandinavia, now host more species-rich, and possibly more abundant, ungulate communities than historically. These changes in ungulate communities are suggested as a major driver of the increase in distribution of *I. ricinus* (Jaenson et al., 2012). Several studies have shown that ungulates – either via their presence or their densities – are associated with a higher density of questing ticks (Dickinson et al., 2020;

Gandy et al., 2021; Gilbert et al., 2012; Hofmeester et al., 2017). Furthermore, tick-borne pathogens have been found to occur in ungulates and ticks feeding on ungulates (e.g., Díaz-Cao et al., 2021; Kazimirova et al., 2018), and an association has been found between deer density and the incidence of Lyme borreliosis in humans (Mysterud et al., 2016). Therefore, ungulate management could be a potential tool to mitigate human and veterinary health risk of tick-borne pathogens. Ungulate management actions could, for example, include hunting, fencing or the introduction of natural predators (Mysterud, 2010). However, if we want to use ungulate management to mitigate health risk, should we treat all ungulate species as one, or should we differentiate among the ungulate species that co-occur? Many of the previous studies that investigated the association between ungulates – either their density or presence – and tick density, treated all ungulate species in their study area as one (e.g., Dickinson et al., 2020; Gandy et al., 2021; Gilbert et al., 2012; Hofmeester et al., 2017). Also, studies that investigated the occurrence of tick-borne pathogens in ungulate species either studied only one species (e.g., Michalik et al., 2012; Overzier et al., 2013; Sgroi et al., 2021) or did not compare different species with each other (e.g., Díaz-Cao et al., 2021; Kazimirova et al., 2018). This shows that current studies on ticks and tick-borne pathogens often treat ungulate species as a black box (the 'ungulate box'). I argue that we need to unravel this box and hypothesize that ungulate species play different roles in the life cycle of ticks and the transmission of tick-borne pathogens because of differences in morphology and behaviour among ungulate species (Chapter 3).

1.5 Scope of the thesis

In this thesis, I open the 'ungulate box' and look more closely at the role that different co-occurring ungulate species may play in the transmission of common tick-borne pathogens. My central hypothesis is that ungulate species differ in the ways they affect tick life cycles, and ultimately, the transmission of tick-borne pathogens. I investigated this hypothesis by quantifying the role of the five most common European ungulate species (fallow deer, roe deer, red deer, moose (*Alces alces*) and wild boar) in the life cycle of ticks and in the transmission of two common tick-borne, and zoonotic, pathogens: *A. phagocytophilum* and *B. burgdorferi* s.l.. I focused on *I. ricinus* as the most common tick species across most of Europe. As part

of my central hypothesis, I posed four questions, which led to the different chapters of this thesis.

1. Previous studies have shown that ungulates can feed *I. ricinus* and several tick-borne pathogens have been isolated from ungulates (Paragraph 1.4). However, how big is the role of ungulates in the life cycle of *I. ricinus* and in the transmission of *A. phagocytophilum* and *B. burgdorferi* s.l. relative to other host species within the host community? (**Chapter 2**)

2. Very few studies have directly compared the role of different ungulate species, co-occurring in the same area, for tick life cycles and for the prevalence of tick-borne pathogens. Comparison of species within the same area is needed to control for other factors that could influence, for example, the tick burden. Therefore, I asked if there is a difference among five prominent European ungulate species that co-exist in the same geographical area in Sweden, both in terms of their tick burden and in the prevalence of *A. phagocytophilum* and *B. burgdorferi* s.l. in the ungulates and in ticks feeding on them? (**Chapter 3**)

3. If we look at the health risk for humans and livestock, an important factor is the number of infected ticks in the vegetation looking for their next bloodmeal. There are three potential ways in which ungulate species may influence this number of questing ticks. Firstly, by providing a blood meal to ticks, allowing the tick to moult into the next life stage or, in case of a female, lay eggs to provide the next generation. Secondly, by their impact on the vegetation and thus on tick habitat, which could influence the tick densities directly. Thirdly, through their impact on the vegetation, ungulates can also affect the densities of other host species, such as small mammals, that play an important role in the life cycle of *I. ricinus* and in the transmission of tick-borne pathogens. Therefore, I asked: do different ungulate species have a different effect on the structure of the field layer vegetation and on the number of questing ticks, infected with tick-borne pathogens or not? And does the composition

of the ungulate community then, as a consequence, matter for the number of (infected) questing ticks? (**Chapter 4**)

4. Species composition and densities of wild ungulate communities are changing in Europe (Apollonio et al., 2010; Spitzer, 2019). But how do these changes relate to the role the ungulates are playing in the life cycle of *I. ricinus* and in the transmission of tick-borne pathogens? And does it matter how the species composition changed for the maintenance and transmission of the different pathogens? (**Chapter 5**)

In **Chapter 6,** I synthesize my findings to these questions and ponder over the question whether ungulate management should indeed be tailored towards specific ungulate species, to be able to mitigate public health risk caused by tick-borne pathogens. I should emphasize here that I only look at ungulate management as a tool to mitigate public and veterinary health risk for tick-borne diseases. There are, however, many other factors that influence the decisions made in ungulate management, such as the effects of ungulates on the forestry industry (e.g., browsing) or their effect on traffic safety (e.g., collisions). Lastly, in **Chapter 7** I discuss questions that have remained unanswered in my thesis and seek further exploration.

2. Ungulates as part of a host community

Before investigating how the different ungulate species affect the life cycle of *I. ricinus* and the transmission of tick-borne pathogens, I looked into the overall role of ungulates in the life cycle of *I. ricinus*, and the transmission of tick-borne pathogens, relative to other host species in the host community. In this chapter, I discuss the importance of the most common European ungulates in general, relative to other vertebrate species, for the life cycle of *I. ricinus* and the transmission of two tick-borne pathogens: *A. phagocytophilum* and *B. burgdorferi* s.l.

2.1 Background

I. ricinus is a generalist parasite that can feed on a multitude of host species (Piesman & Gern, 2004). However, not all vertebrate hosts feed the same number of ticks, and the same life stages of ticks. These differences can be linked to host characteristics, such as host body mass and the densities at which they occur (Hofmeester et al., 2016; Mejlon & Jaenson, 1997; Pfäffle et al., 2013). Descriptive studies have suggested that immature stages feed mainly on smaller vertebrates such as rodents, which occur in higher densities than larger vertebrates (Pfäffle et al., 2013). Adult ticks require a larger blood meal, and feed therefore on larger vertebrates such as ungulates (Pfäffle et al., 2013). Host species do not only differ in their tick burden, but also in their ability to infect ticks with tick-borne pathogens, depending on the pathogen and its ecotype or genospecies. For example, two genospecies of *B. burgdorferi* s.l. are associated with different host species: *B. afzelii* with small mammals and *B. garinii* with birds (Hanincova et al., 2003; Taragel'ová et al., 2008). For the ecotypes of *A. phagocytophilum*, ecotype 1 has been associated with a multitude of host species, including humans,

while the other three ecotypes seem more associated with specific host species: ecotype 2 with roe deer, ecotype 3 with small mammals and ecotype 4 with birds (Jahfari et al., 2014). All this shows that the success of the maintenance of the *I. ricinus* life cycle, and the success of the transmission and maintenance of tick-borne pathogens, depends on the composition of the vertebrate host community. This is the reason why, as part of the meta-analysis in paper I, we constructed a theoretical host assemblage with dominant species in Europe (Table 1) to work with (Box 1b). For each species within this host assemblage, we calculated their relative importance to the *I. ricinus* life cycle and the transmission of *A. phagocytophilum* and *B. burgdorferi* s.l. We did this based on the mean tick burden of these host species and the mean infection prevalence in their feeding ticks, which we obtained through a systematic literature search (Box 1a). Since we used data from this literature search, we were limited in terms of which species we could include in our theoretical host assemblage. Several common species are understudied. These include Eurasian jay (*Garrulus glandarius*), red squirrel (*Sciurus vulgaris*), great spotted woodpecker (*Dendrocopos major*) and fallow deer.

Table 1. Constitution of the theoretical host assemblage with their taxonomic class and the order of magnitude of the density (number per km^2) of occurrence of the host species.

Species		**Taxonomic class**	**Density**[†]
Alces alces	Moose	Ungulate	10^0
Apodemus sylvaticus	Long-tailed field mouse	Small mammal	10^3
Capreolus capreolus	Roe deer	Ungulate	10^1
Cervus elaphus	Red deer	Ungulate	10^0
Microtus agrestis	Field vole	Small mammal	10^3
Myodes glareolus	Bank vole	Small mammal	10^3
Sorex araneus	Common shrew	Small mammal	10^3
Sus scrofa	Wild boar	Ungulate	10^0
Turdus merula	Common blackbird	Bird	10^2
Vulpes vulpes	Red fox	Medium-sized mammal	10^0

[†] Density estimates are obtained from Hörnberg (2001), Niethammer and Krapp (1978) and Cramp and Perrins (1994).

Box 1a. Systematic literature search

Data on *I. ricinus* burden and infection prevalence of *B. burgdorferi* and *A. phagocytophilum* in hosts and feeding *I. ricinus* were collected through two systematic literature searches. The first one was performed by Hofmeester et al. (2016), and contained data on the infection prevalence of *B. burgdorferi* s.l. in vertebrate hosts and feeding *I. ricinus*. Papers published in the period 1945-2014 were included. We supplemented this dataset with data obtained from papers published between January 2015 and August 2021 (Paper IV). The second literature search was performed by us, where we obtained data on the *I. ricinus* burden and the infection prevalence of *A. phagocytophilum* in vertebrate hosts and feeding *I. ricinus* (Paper I). Papers published in the period 1945-2018 were included. We supplemented this dataset with data on *I. ricinus* burden from papers published between January 2019 and August 2021 (Paper IV). Note that the analyses in this chapter are performed as described in Paper I, but with the data obtained by the two supplemented systematic literature searches. The results in Paper I and Chapter 5 are only based on data from the non-supplemented second literature search.

Box 1b. Construction of the theoretical host assemblage

In our theoretical host assemblages, we could only select species for which we obtained the mean tick burden of all life stages and the infection prevalence in hosts from our literature search. Species that are dominant in Western Europe were included, and the order of magnitude of their estimated densities in which they occur were obtained from the literature (Cramp & Perrins, 1994; Hörnberg, 2001; Niethammer & Krapp, 1978). (Paper I)

2.2 Relative importance for feeding *I. ricinus*

The systematic, quantitative, literature review confirmed that different life stages of *I. ricinus* feed on different host groups. Small mammals contributed the most (87%) to feeding larvae of *I. ricinus* (Figure 1A). Birds contributed most (44%) to feeding nymphs, with ungulates (32%) and small mammals (24%) as a close second and third (Figure 1A). Ungulates contributed almost solely (99%) to feeding adult *I. ricinus* (Figure 1A). This data shows that for the maintenance of the life cycle of *I. ricinus*, at least a few host species of different taxonomic groups need to be present in a host community. Since ungulates feed such a large proportion of the *I. ricinus* adults, the presence

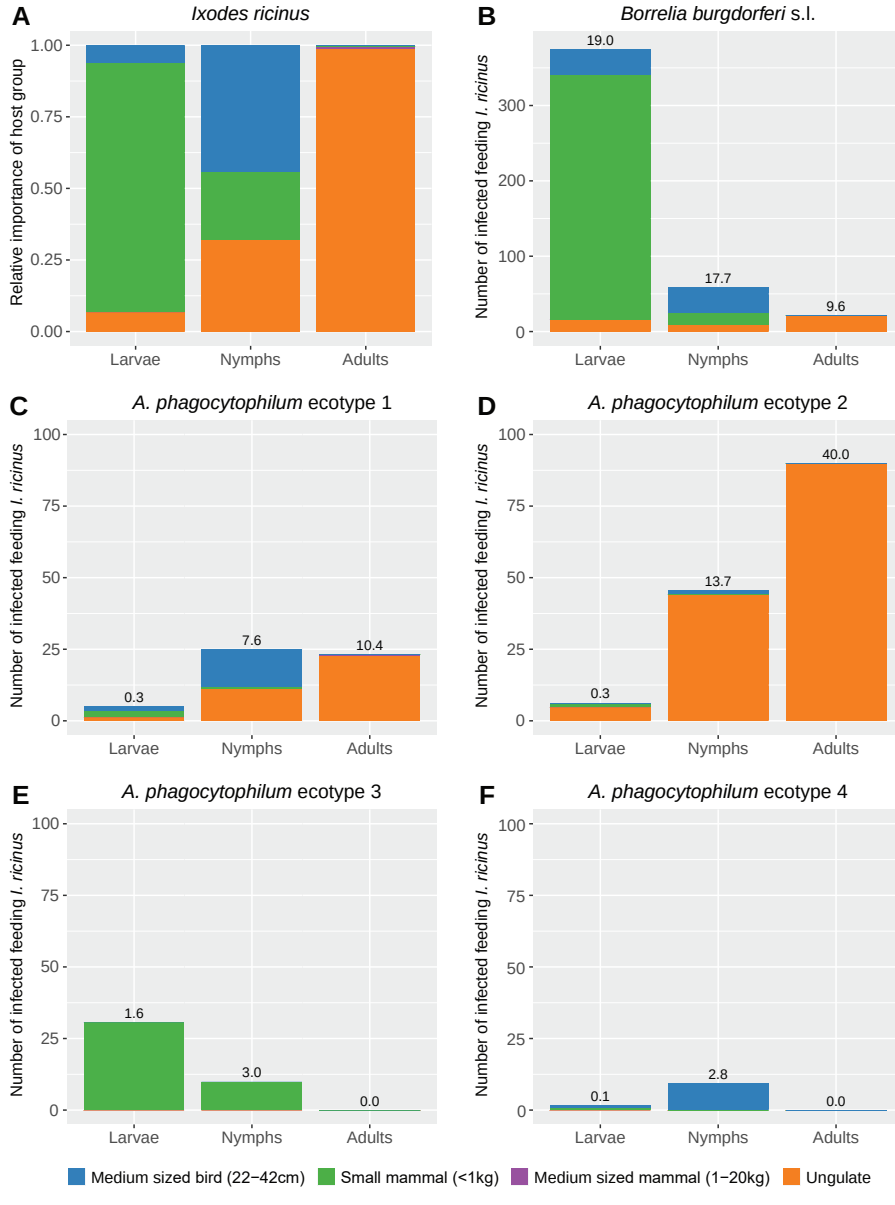

Figure 1. A) Quantification of the relative importance of different host groups in feeding *Ixodes ricinus*. B-F) The expected number of feeding *I. ricinus* infected with *Borrelia burgdorferi* s.l. (B) and the four ecotypes of *Anaplasma phagocytophilum* (C-F) per life stage in our theoretical host assemblage, and the relative contribution of the different host groups to the production of these engorged and infected *I. ricinus*. Values on top of bars represent the percentage of feeding ticks that are infected within the host assemblage.

of ungulates in a host community seems essential for the maintenance of the *I. ricinus* life cycle. Previous studies, where ungulates were excluded from a certain area, confirm this suggestion (Gandy et al., 2021; Hofmeester et al., 2016): when ungulates are not present, the number of questing ticks in the vegetation is reduced. Our results also show that the immature stages of *I. ricinus* feed on smaller hosts than the adult *I. ricinus*, which confirms previous field studies (Reviewed in Pfäffle et al., 2013). Adults feed on larger animals, which is believed to be because they require a larger blood meal than the immature stages (Pfäffle et al., 2013). For this reason, they quest higher to be able to select for the larger animals. The immature stages cannot quest at the same height as adults, since they are smaller and are thus more prone to dehydration (Mejlon & Jaenson, 1997). Ticks rehydrate in the soil, and therefore the immature stages need to stay closer to the ground (Mejlon & Jaenson, 1997). At this height, they are more likely to encounter smaller hosts. In addition, smaller hosts usually live at higher densities than larger hosts, also explaining why immature stages are more likely to feed on smaller hosts.

2.3 Relative contribution to transmission of pathogens

To calculate the relative importance of different host species for the transmission of either *A. phagocytophilum* or *B. burgdorferi* s.l., we looked at how many infected engorged ticks of each life stage the different host species carried. These ticks could have become infected while feeding on that host species, or while feeding as an earlier life stage on the same or a different host species. Only larvae must have become infected during their feeding on the host species they were found on, because questing larvae are thought to be uninfected with *A. phagocytophilum* and *B. burgdorferi* s.l. due to the absence or inefficiency of transovarial transmission of these pathogens in *I. ricinus* (Hauck et al., 2020; Richter et al., 2011).

2.3.1 Anaplasma phagocytophilum

The relative importance of the different host species in the transmission of *A. phagocytophilum* depends on its ecotype. For ecotype 1, the relative importance of the different taxonomic groups varied among the life stages of *I. ricinus*, while ungulates, small mammals and birds dominated the relative importance for ecotype 2, 3 and 4, respectively (Figure 1C-F). It should be

noted, however, that few of the papers in our systematic literature search reported the ecotype of the ticks positive for *A. phagocytophilum* and, therefore, we had to estimate the ecotype based on literature by Jaarsma et al. (2019). This means that the distribution of ecotypes among the host species in our study is very similar to the distribution of the ecotypes among the positive samples in Jaarsma et al. (2019). We can, however, not rule out that ecotypes have different species-specific transmission rates, and thus that the contribution of the various host species as reported in Figure 1C-F can be slightly different in reality. Furthermore, our study only focussed on *I. ricinus* and we cannot exclude the role of other tick species in the circulation of ecotype 3 and 4. Bown et al. (2008) showed a correlation between the *A. phagocytophilum* infection prevalence in field voles and the *Ixodes trianguliceps* burden. Since small mammals are associated with ecotype 3, this could indicate that *I. trianguliceps* also plays a role in the circulation of this ecotype. Ecotype 4 has been found in *Ixodes frontalis* and *Ixodes ventalloi* (Jaarsma et al., 2019), and these tick species might therefore play a role in the circulation of ecotype 4.

For all ecotypes combined, it was clear that ungulates produced the majority of the engorged ticks that tested positive for *A. phagocytophilum* (Figure 1C-F). This finding confirms previous, more qualitative, reviews that showed that *A. phagocytophilum* has been detected in many ungulate species worldwide (Stuen et al., 2013). In Europe, the density of red and fallow deer, and to a lesser degree roe deer, determine the density of questing ticks positive for *A. phagocytophilum* (Takumi et al., 2021). In North America, white-tailed deer seem to be one of the major reservoir hosts for *A. phagocytophilum* (Massung et al., 2005). Our data also shows that *I. ricinus* mainly become infected as nymphs or as adults. The infections of feeding adults seem to be irrelevant for the circulation of *A. phagocytophilum*, since transovarial transmission is absent (Hauck et al., 2020). Our data supports the hypothesis that the circulation of *A. phagocytophilum* is mainly between nymphs and adults (Takumi et al., 2019). Due to the high relative importance of ungulates for feeding the adult stage (Figure 1A) only a few immature *I. ricinus* that become infected is sufficient to maintain a relatively high infection prevalence in ungulates (See Appendix S2 of Paper I).

2.3.2 *Borrelia burgdorferi* s.l.

The relative importance of the different host species in the transmission of *B. burgdorferi* s.l. also varied among the life stages of *I. ricinus*. Small mammals produce the majority of the infected engorged larvae, while birds and ungulates dominate the production of the infected engorged nymphs and adults, respectively (Figure 1B). It is important to note, however, that the number of *I. ricinus* infected with *B. burgdorferi* s.l. drastically declines from larvae to nymphs to adults (Figure 1B). This means that over all life stages combined, small mammals contribute the most to production of engorged ticks infected with *B. burgdorferi* s.l., while the contribution of ungulates seems to be negligible. This is in line with previous studies on ungulates which shows that ungulates, both in Europe and in North America, are inefficient reservoirs (Jaenson & Tälleklint, 1992; Kurtenbach et al., 2002; Matuschka et al., 1993; Telford et al., 1988). It even has been proposed that blood from ungulates has a borreliacidal effect, i.e., it possibly kills the bacteria that are present in the tick when the tick feeds on ungulates (Pacilly et al., 2014). Based on our results we can, however, not conclude that ungulates merely have a negative or neutral contribution to the transmission cycle of *B. burgdorferi* s.l. We showed that adult *I. ricinus* mainly feed on ungulates, and ungulates are therefore key species in the maintenance of the life cycle of *I. ricinus*. A functioning life cycle is important in the transmission cycle of tick-borne pathogens. Ungulates are therefore also likely to play a role in the transmission cycle of *B. burgdorferi* s.l., even though they hardly carry any ticks infected with this pathogen.

2.4 Conclusion

Our systematic review showed that ungulates play an important role in the life cycle of *I. ricinus*, by feeding adults, and in the transmission of *A. phagocytophilum*, although the extent of their role depends on the ecotype. The contribution of ungulates to the circulation of *B. burgdorferi* s.l. is more complicated. Based on the number of engorged ticks on ungulates infected with *B. burgdorferi* their contribution seems to be negligible. Through their possible borreliacidal effect this contribution can be seen as negative. However, by feeding adult *I. ricinus* ungulates may play an important role in maintaining the life cycle of *I. ricinus* and as such contribute to the transmission of tick-borne pathogens. With our study we also see that the

whole host community is important in the maintenance of the life cycle of *I. ricinus*. For pathogens where ungulates are not the only maintenance hosts, the composition of the entire host community can affect the success of the transmission of the pathogen (Chapter 5). I believe that the general results of this study are robust, even though the exact numbers and importance of the individual host species might differ between geographical areas because of many factors, including the interactions among host species and indirect effects of hosts on tick populations.

In this chapter I have shown that ungulates do play an essential role in the life cycle of *I. ricinus* and the transmission of mainly *A. phagocytophilum*, but also *B. burgdorferi* s.l.. It is thus useful to open the 'ungulate box', and look into the role of different ungulate species, which I will do in the following chapters.

3. Ungulates as hosts for *Ixodes ricinus*

In Chapter 2, I showed that ungulates as a group play a major role in the life cycle of *Ixodes ricinus* and in the transmission of tick-borne pathogens. There are, however, many ungulate species worldwide and all differ morphologically and behave differently. It is obvious that ungulate species like a giraffe (*Giraffa camelopardalis*) and a killer whale (*Orcinus orca*) are completely different from each other. However, even ungulate species that co-occur within the same host community, and that seem very similar on the surface (e.g., diverse deer species) may have very different morphological and behavioural traits. These traits, such as feeding type, social behaviour, or body mass, may affect the ability of ungulate species to feed ticks and transmit tick-borne pathogens. In this chapter, I show how five common ungulate species (fallow deer, roe deer, red deer, moose, and wild boar), co-occurring in south-central Sweden, differ in their tick burden and infection prevalence of tick-borne pathogens. Furthermore, I discuss how morphological and behavioural differences among these five ungulate species could explain these differences.

3.1 Ungulates as propagation hosts

In Chapter 2, I established that adults feed mainly on ungulate species, which indicates that ungulates act as propagation hosts for *I. ricinus*. These are hosts that are needed to ensure the reproduction of *I. ricinus*. The mating of *I. ricinus* can occur both on- and off-host, although a bloodmeal is necessary for females to reproduce (Kiszewski et al., 2001). In Paper II, we collected feeding females and non-feeding males from fallow deer, roe deer, red deer, moose, and wild boar that hunters shot on hunting estates in south-central Sweden (Box 2). For all ungulate species we determined their infestation prevalence (i.e., the proportion of individuals infested with ticks) and their

Box 2. Investigating the role of ungulates in the propagation of I. ricinus and in the transmission of tick-borne pathogens.

We opportunistically collected ticks and spleens from fallow deer, moose, red deer, roe deer and wild boar, that were shot in south-central Sweden during the moose hunting seasons of 2018 and 2019 (Figure 2). We counted the number of ticks separately for ears, head, neck, axilla, groin, front legs, hind legs, and the rest of the body. Furthermore, we collected questing nymphs and adults by dragging a $1m^2$ white cotton cloth in the same area as where the ungulates were shot. We tested the ticks, both questing and feeding, and spleens for the presence of *A. phagocytophilum* and *B. burgdorferi* s.l. with qPCR (According to Heylen et al., 2013; Stigum et al., 2019). We calculated, per ungulate species, the infestation prevalence (i.e., proportion of individuals infested with *I. ricinus*) for feeding larvae, nymphs, females, and non-feeding males. Furthermore, we calculated the infestation intensity (i.e., average number of ticks per individual if the individual was infested) for feeding larvae, nymphs and females, and the infection intensity (i.e., average number of infected ticks per animal) of larvae, nymphs, and females for *A. phagocytophilum* and *B. burgdorferi* s.l. We compared the infestation intensity, infestation prevalence and infection intensity in feeding larvae among the different ungulate species using a Šidák-adjusted Dunn-test. For the other life stages of *I. ricinus*, we compared the infestation intensity, infestation prevalence and infection intensity among the different ungulate species using hierarchical generalized linear mixed models (GLMMs) with binomial and zero-truncated distributions. (Paper II)

Figure 2. The collection of ticks from shot ungulate species.
Pictures by Jimmy Pettersson and Kas Swinkels.

infestation intensity (i.e., the average number of ticks on an infested host). The deer species and wild boar differed clearly in terms of whether animals were infested with *I. ricinus* adults or not (Figure 3E&G). Deer species were 32 times more likely to be infested with feeding females than wild boar. Deer were also more often infested with non-feeding males than wild boar. Due to the low number of feeding females on wild boar, we could not determine the infestation intensity for wild boar. Both the infestation intensity and infestation prevalence of feeding females did not differ among the different deer species (Figure 3E-F). We did find that roe and fallow deer were less often infested with non-feeding males than moose (Figure 3G). Our numbers of non-feeding males were small though and these results need to be interpreted with care. Overall, differences among deer species in adult infestation were relatively small, but wild boar seems to play a minor role as propagation host relative to the deer species.

3.2 Ungulates as hosts for immature stages

The ungulates we studied in Paper II did not only feed mature stages of *I. ricinus*, but also immature stages. These stages were collected from the shot ungulates in Paper II as well. Fallow deer were more likely to feed *I. ricinus* larvae than red deer and wild boar (Figure 3A). The numbers of larvae we found on all ungulates were small though, and these results should therefore be interpreted carefully. Furthermore, we found that fallow deer and roe deer were more likely to feed *I. ricinus* nymphs than the other ungulate species, of which wild boar had the lowest infestation prevalence (Figure 3C). We did not find any differences among fallow deer, red deer, and roe deer in their infestation intensity for both larvae and nymphs (Figure 3B&D). Due to the low number of feeding larvae and nymphs on wild boar and moose, we could not determine the infestation intensity of the immature stages for these species. For both the immature and the mature stages, we found a lower infestation than in previous European studies (e.g., Pacilly et al., 2014; Tälleklint & Jaenson, 1994; Wegner et al., 1997). The reason for this might be geographical. Aspects like climate, vegetation and general mammal density might be different in our study area than elsewhere. Also, we were restricted to the moose hunting season for our sampling, and since this is at the end of the tick season this could also explain our relatively low infestation levels.

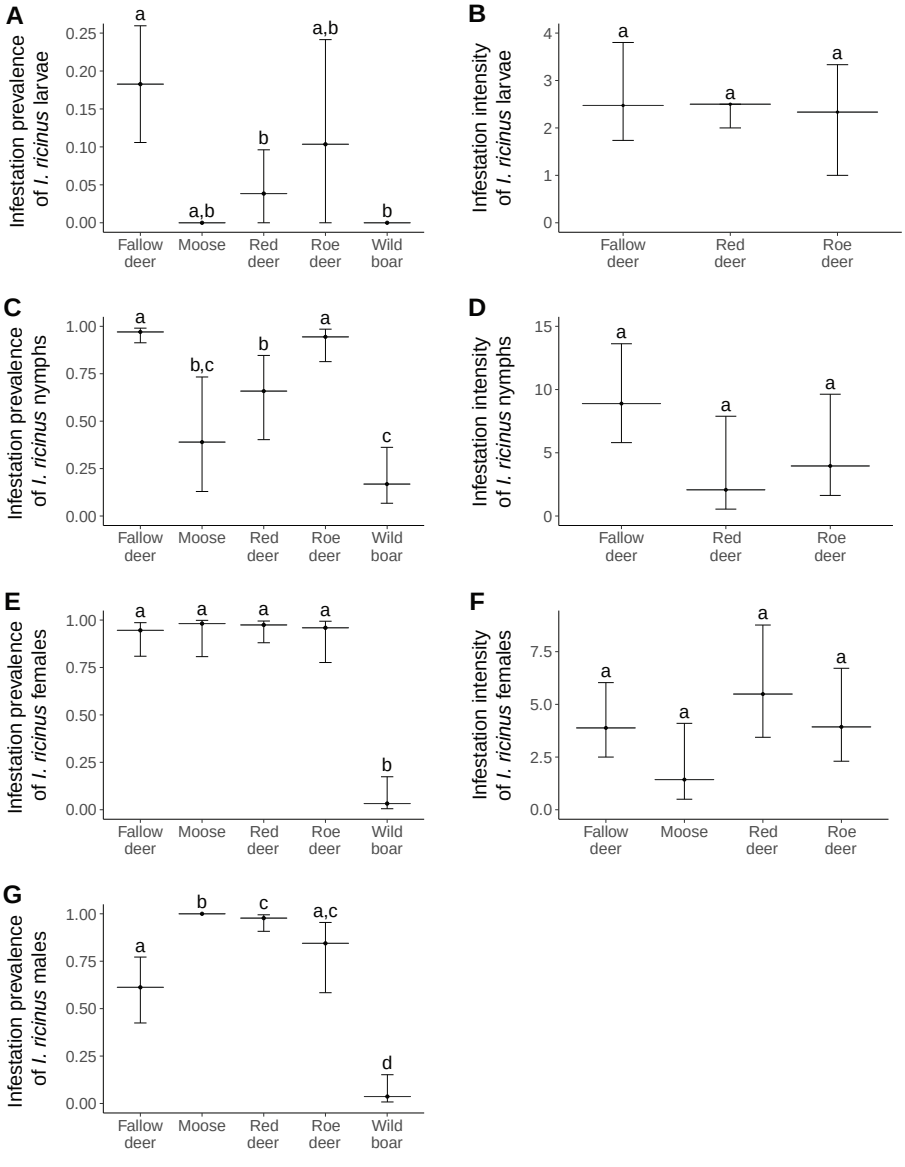

Figure 3. Infestation prevalence of feeding *I. ricinus* larvae (A), feeding nymphs (C), feeding females (E) and non-feeding males (G). Infestation intensity of feeding *I. ricinus* larvae (B), feeding nymphs (D) and feeding females (F). All values are given with 95% bootstrapped, bias-corrected, confidence intervals. Lowercase letters indicate the significant difference among the ungulate species. All values are values predicted from the models in our study, except for the larvae (Box 2).

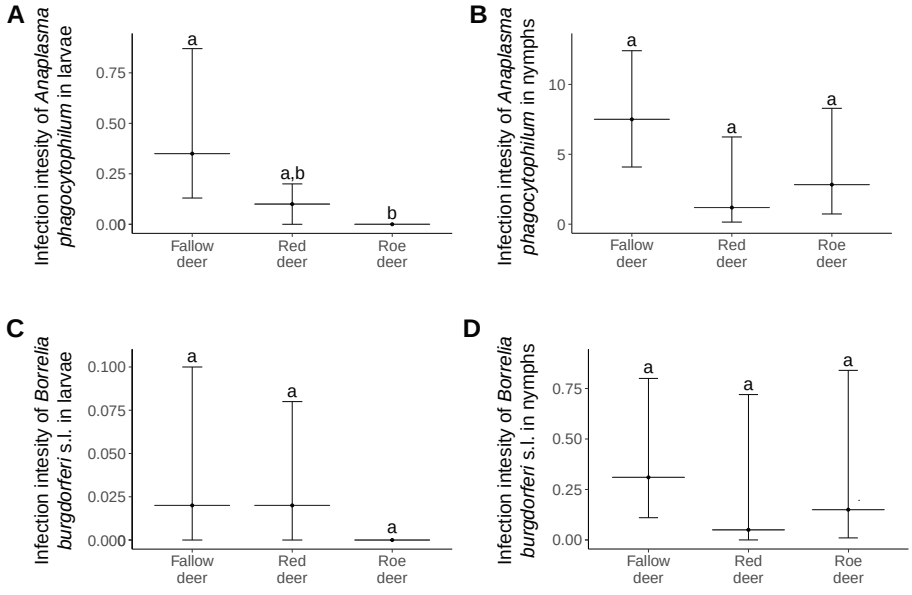

Figure 4. Infection intensity of *Anaplasma phagocytophilum* in feeding *I. ricinus* larvae (A) and nymphs (B) and of *Borrelia burgdorferi* s.l. in feeding *I. ricinus* larvae (C) and nymphs (D). All values are given with 84% bootstrapped, bias-corrected, confidence intervals. Lowercase letters indicate the significant differences among the ungulate species. The infection intensities in feeding nymphs for both pathogens are the values predicted from the models in our study (Box 2).

3.3 Role of ungulates in transmission of pathogens

To investigate the difference among the ungulate species in terms of their role in the transmission of tick-borne pathogens, we calculated the infection intensity (i.e., the total number of infected ticks on an average individual of a certain host species) per ungulate species. To do so, we tested the collected feeding larvae and nymphs from the shot ungulates for *A. phagocytophilum* and *B. burgdorferi* s.l. (Box 2). Adult *I.* ricinus were not considered in this part of the study, because they do not produce any offspring infected with either *A. phagocytophilum* or *B. burgdorferi* s.l. (Hauck et al., 2020; Richter et al., 2011; van Duijvendijk et al., 2016). Since we only collected a few feeding larvae and nymphs from moose and wild boar, we could not calculate the infection intensity for these species. The infection intensity of *A. phagocytophilum* in larvae feeding on fallow deer was higher than for roe deer, but for nymphs we did not find a difference among roe, fallow and red

deer (Figure 4A-B). This difference among the ungulate species can be traced back to the differences among the ungulate species in their infestation prevalence of feeding *I. ricinus* larvae (Figure 3A), since the infection intensity is calculated with the infestation prevalence (See Equation 2 in Paper II). For the infection intensity of *B. burgdorferi* s.l. in both larvae and nymphs, we did not find any difference among the ungulate species (Figure 4C-D). Notably, the infection intensity for *A. phagocytophilum* was a lot higher than the infection intensity for *B. burgdorferi* s.l. in both larvae and nymphs (Figure 4).

3.3.1 Pathogen transmission from ungulates to ticks

In Paper II, we not only collected *I. ricinus* from ungulates, but we also collected questing nymphs and adults, and tested these for the presence of *A. phagocytophilum* and *B. burgdorferi* s.l. Since the questing ticks came from the same area as the ungulates, comparing the infection prevalence in questing ticks with the infection prevalence in the feeding ticks can give insight into whether the ungulates can transmit the pathogen to the tick. We found that for all deer species, the *A. phagocytophilum* infection prevalence was lower in questing nymphs and adults than in feeding nymphs and adults. This is an indication that deer, but not wild boar, are important transmission hosts for *A. phagocytophilum*, as has been suggested before (Stuen et al., 2013; Takumi et al., 2021). We also tested spleens from the different host species for the presence of tick-borne pathogens, and we found a high infection prevalence of *A. phagocytophilum* in all ungulate species: 0.71 in wild boar, 0.98 in fallow deer and 1.00 in moose, red deer and roe deer. There was, however, a clear difference between roe deer and the other ungulate species; roe deer only harboured *A. phagocytophilum* ecotype 2, while the others only harboured ecotype 1. This is in line with previous studies (Jaarsma et al., 2019; Jahfari et al., 2014; Stigum et al., 2019) and indicates that roe deer are important transmission hosts for the non-zoonotic *A. phagocytophilum* ecotype 2, while the other deer species are important transmission hosts for the zoonotic ecotype 1.

We found that the infection prevalence of *B. burgdorferi* s.l. was either higher or similar in questing nymphs and adults than in feeding nymphs and adults from all ungulate species. This indicates that none of the ungulate species are transmission hosts for *B. burgdorferi* s.l., which is underlined by

the fact that none of the spleens from ungulates tested were positive for *B. burgdorferi* s.l..

3.4 Morphological and behavioural differences

We showed that all deer species we studied have a similar role as propagation hosts in the life cycle of *I. ricinus* (Figure 5). Wild boar on the other hand played a negligible role as propagation host (Figure 5). In the transmission of *A. phagocytophilum*, all deer species played a role, although this role seemed to be slightly different for each species (Figure 5). Among the deer species we did not find any differences in their role in the transmission of *B. burgdorferi* s.l. (Figure 5). The role of wild boar is negligible in the transmission of both *A. phagocytophilum* and *B. burgdorferi* s.l. (Figure 5). The observed differences in the role in the life cycle of *I. ricinus* and in the transmission of the investigated tick-borne pathogens provide initial support for our hypothesis that morphological and behavioural traits might affect their ability to feed *I. ricinus* and play a role in the transmission of tick-borne pathogens.

Our data showed that wild boar feed significantly fewer ticks than the deer species. This could be because it is harder for ticks to attach to a wild boar and/or it is harder for ticks to stay attached. For example, the dense hair structure and thick skin of wild boar could influence the potential for ticks to attach and find a blood meal. Moreover, the wallowing behaviour of wild boar could make it harder for ticks to stay attached. We did not find any significant differences among the deer species in their ability to feed female *I. ricinus*. However, we did find that moose feed fewer females, but this was not significantly different from the other deer species, possibly due to the low number of moose sampled (Figure 3F). A potential lower number of females feeding on moose might be explained by the length of the legs. The majority of the female *I. ricinus* are found feeding in the axilla or the groin. This has been found by us (See Table S2 of Paper II) but also by previous studies (Kiffner et al., 2011; Pacilly et al., 2014). The length of the leg therefore determines the distance a tick has to travel from the point on the leg where it encounters the host to the groin or axilla. Since moose have longer legs than the other deer species, this distance is therefore also longer. Among the deer species, we saw a difference in the infestation prevalence of

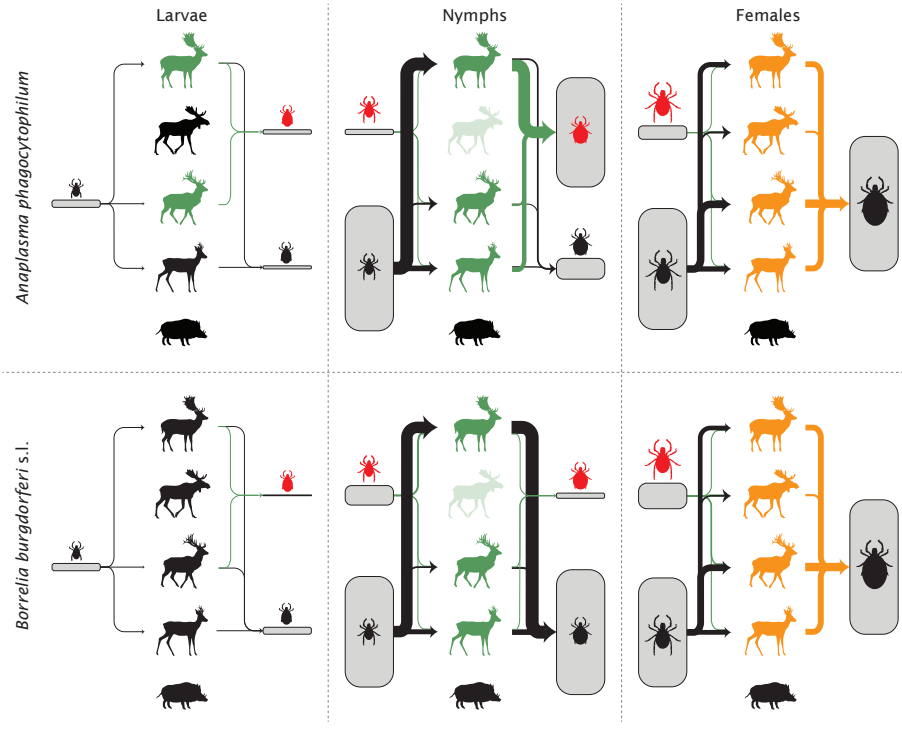

Figure 5. Illustration of the transmission of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. by ungulate species. The arrows from questing ticks to ungulates show the attachment routes and the arrows from ungulates to engorged ticks show detachment routes. The thickness of the arrows represents the proportion of ticks attaching or detaching, and the size of the boxes represents the proportion of that tick stage, based on data from our study. Red ticks represent infected ticks. The green arrows and green ungulates show the role of the ungulate species in the transmission of either *A. phagocytophilum* or *B. burgdorferi* s.l., and the orange arrows and orange ungulates show the role of the ungulate species as propagation hosts. Light-green coloration of ungulates means that the role of this ungulate in the transmission of the relevant pathogen is unknown. Silhouettes of ungulates by Sander Vink, and silhouettes of ticks by Tim Hofmeester.

I. ricinus larvae, where fallow deer were more likely to feed larvae. Larvae were only found on the ears of the ungulates (See Table S2 of Paper II), and we hypothesize that ungulate feeding behaviour might be important in this context. Fallow deer feed predominantly in the field layer, which makes it easier for larvae, but also nymphs, to attach to the ears of fallow deer than to

ears of species that browse higher up, such as moose (Spitzer et al., 2020). We could even argue that the skin on the ears is quite thin, which makes it easier for the small immature stages to find a blood meal.

There are many morphological and behavioural traits that could influence the infestation prevalence and intensity of the different ungulate species, even more than the few mentioned here. Different traits within the same species could even have opposite effects on tick infestation, and all traits combined determine the tick burden of the host species. However, with the data that we collected we can only say that there are differences in infestation prevalence and intensity among the ungulate species, and not which traits determine these differences. Furthermore, the tick burden is not only driven by characteristics of the hosts, but also external factors like the tick density, which is driven by the complete host community composition, and (micro)climatic factors. We did find some differences in the infection intensity of *A. phagocytophilum* in feeding larvae among the ungulate species. These differences can, however, be explained by the differences in infestation prevalence among the ungulate species. We therefore hypothesize that the morphological and behavioural traits discussed before could also drive the differences in infection intensity we found.

3.5 Conclusion

The study in Paper II supported my hypothesis that different ungulate species have different roles in the propagation of *I. ricinus* and in the transmission cycles of tick-borne pathogens. This study, however, only compared the different ungulate species with each other, and therefore I can only draw conclusions on the relative contribution of the five ungulates studied. To assess how these contributions relate to the whole host community, other important host species should also be included. The next question concerns the different roles of the ungulate species in the propagation of *I. ricinus* and how this role in the transmission cycles of tick-borne pathogens relates to the number of (infected) questing ticks. This I will discuss in the next chapter.

4. Ungulates and questing *Ixodes ricinus*

In the previous chapters, I have shown that ungulates play a role in the life cycle of *I. ricinus* and in the transmission of tick-borne pathogens, but that this role differs among the different ungulate species. Now I ask myself, do the differences in the role in the life cycle of *I. ricinus* and in the transmission of tick-borne pathogens, also affect the number of (infected) questing ticks? Ungulates may affect the number of (infected) questing ticks through two mechanisms (Figure 6): indirectly, via their effect on the field layer vegetation, and directly, by providing a blood meal. Ungulates can shape the field layer vegetation through, for example, grazing, browsing, trampling, defecating, and seed dispersal (Gill & Beardall, 2001; Ramirez et al., 2018). This could affect the microclimate and questing possibilities for ticks (Daniel et al., 1977), but can also change food availability and protection against avian predators for small mammals (Ecke et al., 2002), which are also important hosts in the life cycle of *I. ricinus* (Chapter 2). In this chapter, I investigate whether the ungulate community composition affects the number of (infected) questing ticks along known gradients of densities of different ungulate species via the indirect and direct mechanisms.

4.1 Effect of ungulates on field layer vegetation

Feeding behaviour of the different ungulate species might influence their effect on the height of the field layer. Both red and fallow deer are intermediate feeders and have large amounts of grasses and forbs in their diet (Spitzer et al., 2020). I hypothesize that these species therefore have a stronger negative effect on the height of the field layer, than species like moose and roe deer whose diet is more dominated by woody plant species (Spitzer et al., 2020). Wild boar graze during the growing season and their

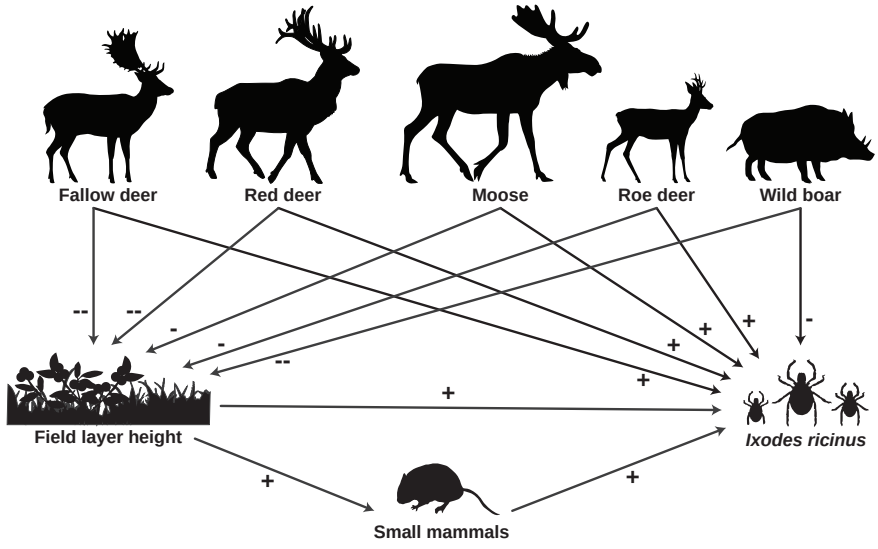

Figure 6. Schematic representation of how I hypothesize that the different ungulate species are associated with the field layer height, small mammal abundance and *Ixodes ricinus* density. + indicates a positive association, - indicates a negative association and -- indicates a strong negative association. Silhouettes of ungulates, field layer, ticks and small mammal made by Sander Vink, Nannet Fabri, Tim Hofmeester and Frauke Ecke, respectively.

rooting behaviour can temporarily remove the field layer. In addition to fallow and red deer, wild boar can thus have a strong negative effect on the height of the field layer. To investigate whether ungulate species differ in terms of their effect on the field layer vegetation, we performed a field study (Paper III) in south-central Sweden on transects along gradients of the densities of five ungulate species: fallow deer, roe deer, red deer, moose, and wild boar. On each of these transects we determined the ungulate abundance (as expressed by passage rates in front of camera traps) and the height of the field layer (Box 3). As hypothesized, a lower mean height of the field layer was associated with a higher density of fallow deer (Figure 7). However, the field layer height was not associated with densities of any of the other ungulate species. In our study area, fallow deer occur in much higher densities than the other ungulate species, which could explain why the effect on the field layer was more pronounced for fallow deer than for other ungulate species, particularly red deer. For wild boar we did not find the hypothesized association, which could perhaps be explained by the fact that

we only conducted our study in areas where the field layer was present and we thus avoided areas with active rooting behaviour. Previous studies, where ungulates are excluded from certain areas, showed that the presence of ungulates reduces the height of the field layer vegetation (Gandy et al., 2021; Thomann et al., 2018). We not only tested for an association between the separate ungulate species and the field layer height, but we also combined the passage rates of all the ungulate species (Paper III). Here, we did not find an association between the field layer height and the overall ungulate densities. This shows that by treating all ungulate species as one group, the effect of fallow deer is overshadowed by the other species. This result argues in favour for differentiating among the ungulate species instead of treating all species as one group. In our study, we have only looked at the effect of ungulates on the height of the field layer vegetation, since we expected that this would be the strongest effect that ungulates have on the vegetation. However, ungulates could also affect other vegetation factors. The presence of ungulates has been found to have a negative effect on the abundance of woody understorey, saplings, shrubs and mosses, but a positive effect on the species richness of, for example, forbs and mosses (Reviewed in Bernes et al., 2018).

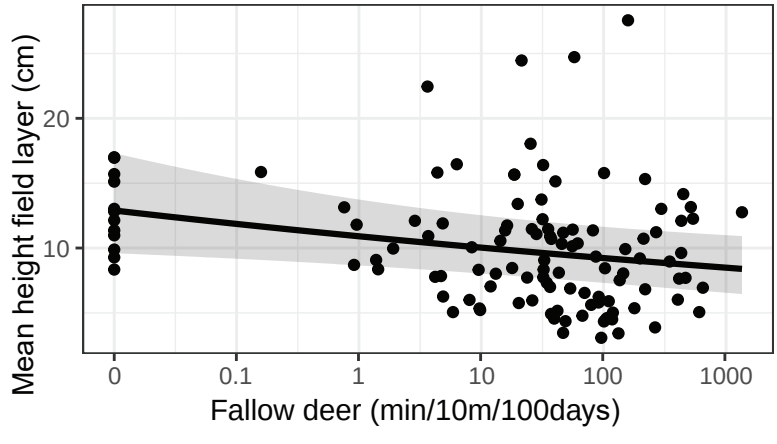

Figure 7. Predicted association between the mean field layer height and the passage rates of fallow deer in a generalized linear mixed model with gaussian distribution (p = 0.011). Grey shading illustrates the 95% confidence interval.

Box 3. Investigating the direct and indirect effect of ungulates on the presence of (infected) I. ricinus in the vegetation.

We established 20 1x1km transects in the area between Gnesta and Nyköping in south-central Sweden, representing as strong as possible gradients of densities of the different ungulate species based on pellet counts performed on these transects during March-April 2016, 2017, and 2018 as part of a different project (Spitzer et al., 2021). On eight points, distributed evenly along each 1x1km transect, we collected ticks by dragging a $1m^2$ white cloth over the field layer vegetation (Figure 8A). We recorded the temperature above the field layer during dragging and measured the height of the field layer (max. 50cm) using the drop disc method (Figure 8B-C; Stewart et al., 2001). To estimate the local variation in ungulate abundance, we used camera traps (Figure 8D) on each point to assess passage rates as the number of minutes per 10 minutes per 100 days in front of the camera (Figure 9). At each point, we also trapped small mammals to estimate the small mammal abundance (Figure 8E). All data were collected during summer 2019. Collected ticks were tested for the presence of *A. phagocytophilum* and *B. burgdorferi* s.l. with qPCR. To test for any effects of ungulate passing rates on the height of the field layer and/or on the presence of *I. ricinus*, we used generalized linear mixed models (GLMMs) with gaussian and binomial distributions. In the GLMM where we tested for any effects of ungulate passing rates on the presence of *I. ricinus*, we also included the temperature above the field layer and the mean height of the field layer. (Paper III)

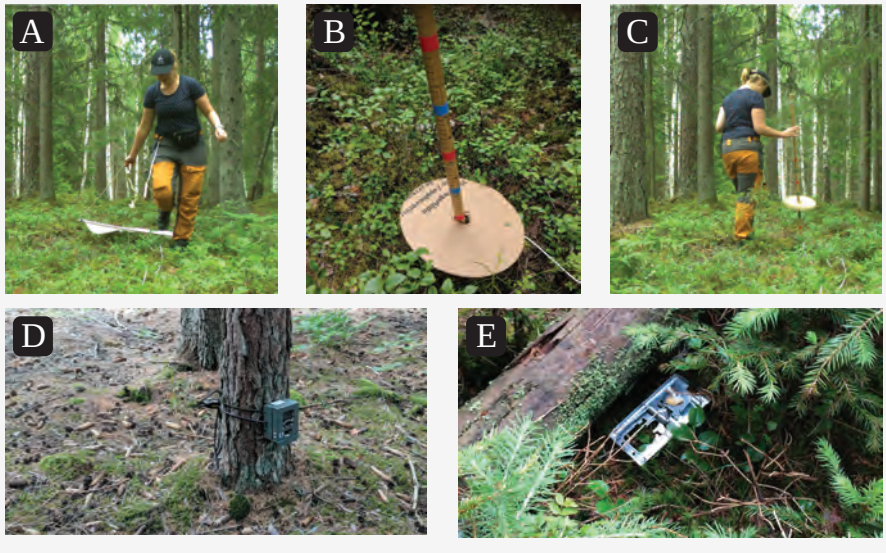

Figure 8. A) The collection of ticks. B-C) The drop disc method to measure the field layer height. D) A camera trap to capture ungulates. E) A snap trap to catch small mammals.

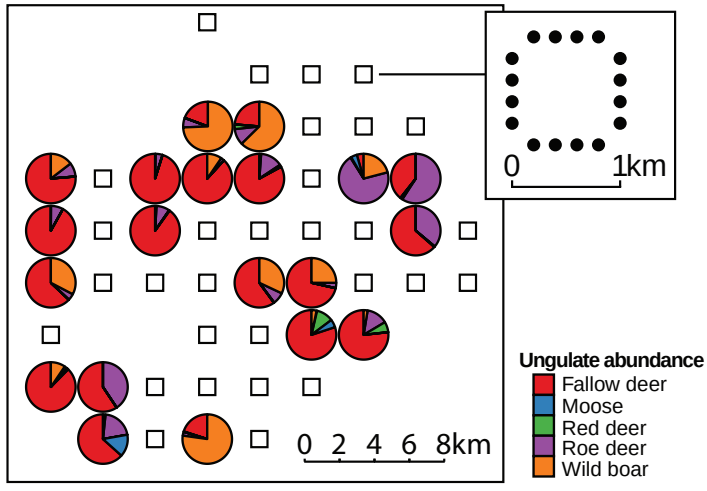

Figure 9. The ratio of the passage rates of the five ungulate species on the 20 studied transects in our study site in south-central Sweden.

4.2 Effect of ungulates on *Ixodes ricinus* density

Gandy et al. (2021) proposed two, not mutually exclusive, mechanisms through which ungulates can affect the density of questing ticks. Firstly, a higher density of ungulates feeds more female ticks, which would lead to a larger density of questing ticks in the next generation. This mechanism is similar to the direct mechanism I proposed earlier in this chapter. Based on the results from Chapter 3, we expected that the direct effect of deer on the *I. ricinus* density in the vegetation would be positive, while the effect of wild boar would be negative. The second mechanism proposed by Gandy et al. (2021) is that the grazing pressure of ungulates would lead to a lower field layer vegetation and a lower density of rodents, resulting in lower densities of *I. ricinus*. This mechanism is comparable with my indirect mechanism. To test if there are any effects of ungulates on the presence of *I. ricinus*, either direct or indirect, we determined the presence of *I. ricinus* in the vegetation on the transects by dragging and recorded the temperature above the field layer (Box 3). We could not detect any effect of the passage rates of the ungulate species on the presence of questing *I. ricinus* of any life stage. We did find that *I. ricinus* adults were more likely to be present at lower temperatures (Figure 10).

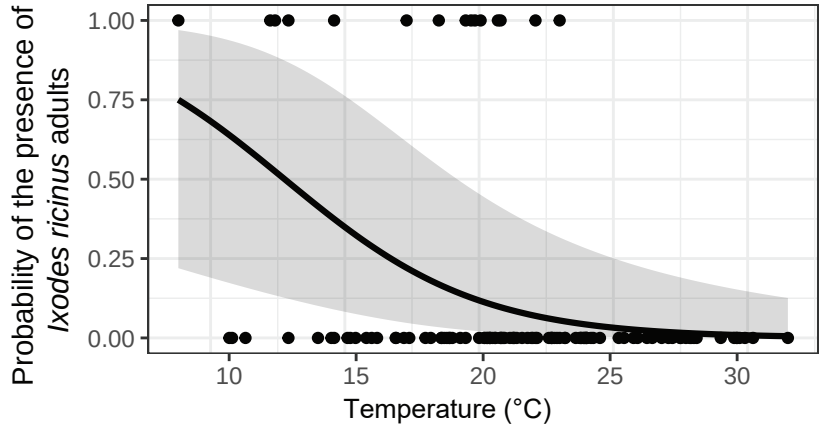

Figure 10. Predicted association between the presence of *I. ricinus* adults and the temperature above the field layer in a generalized linear mixed model with binomial distribution (p = 0.004). Grey shading illustrates the 95% confidence interval.

We expected that ungulates had a positive effect on the *I. ricinus* density via the direct mechanism (i.e., by feeding *I. ricinus*), but that they had a negative effect via the indirect mechanism (i.e., by their effect on the vegetation). These two mechanisms have thus an opposite effect, and can potentially cancel each other out. This might explain why we did not find any associations between the presence of questing *I. ricinus* and the passage rates of ungulate species. There are, however, also other explanations for not finding support for our hypotheses. We found a relatively low density of questing *I. ricinus* in our study area compared to other European studies (Dobson et al., 2011; Heylen et al., 2019; van Gestel et al., 2021). Furthermore, it could be that for some ungulate species that occur in low densities in our study, like red deer and moose, our trapping effort was not high enough. The combination of both low densities of questing *I. ricinus* and of some ungulate species could explain the lack of associations between the presence of questing *I. ricinus* and the passage rates of ungulate species. Due to their rooting behaviour, we expected that wild boar would have a strong negative effect on the presence of questing *I. ricinus*. That we did not find such an association could again be explained by the fact that we only conducted our study in areas with established field layer vegetation, and excluded areas with active rooting.

In our study (Paper III), we looked at associations between the presence of questing *I. ricinus* and the passage rates of ungulate species, and we did not take the densities of questing *I. ricinus* into account, due to the low number of questing *I. ricinus* found. Previous studies have shown that the density of questing *I. ricinus* nymphs is positively associated to either the presence or density of deer, and negatively associated with the density of wild boar (Dickinson et al., 2020; Gandy et al., 2021; Gilbert et al., 2012; Hofmeester et al., 2017; Vourc'h et al., 2016). Furthermore, the density of *I. ricinus* has also been found to be associated with several aspects of the vegetation structure that we did not consider in our study. These aspects are, for example, the main tree species and the cover of the shrub layer (vegetation with a height of 50cm - 3m) (Tack et al., 2012a; Tack et al., 2012b; Vourc'h et al., 2016).

4.3 Effect of ungulates on pathogens in questing ticks

In our study in Paper III, we also aimed to investigate whether the ungulate community composition influenced the density of questing ticks infected with *A. phagocytophilum* or *B. burgdorferi* s.l.. Takumi et al. (2021) showed that the densities of fallow deer and red deer (and to some extent roe deer) drive the number of questing nymphs infected with *A. phagocytophilum*. Based on this, and on the results of Chapter 3, we hypothesized that the density of roe deer would be associated with the number of questing ticks infected with *A. phagocytophilum* ecotype 2, while the densities of other deer species would be associated with the number of questing ticks infected with ecotype 1. Based on the results of Chapter 3 and on what previous studies have found (e.g., Vourc'h et al., 2016), we did not expect that any of the ungulate species would be associated with the number of questing ticks infected with *B. burgdorferi* s.l.. I do want to emphasize, however, that for both pathogens, other vertebrate hosts, like small mammals, could alter the effect of ungulates, since these hosts could also play an important role in the life cycle of *I. ricinus* and the transmission of tick-borne pathogens (Chapter 2). Unfortunately, due to the low number of ticks collected in our study, and consequently a low number of ticks infected with *A. phagocytophilum* and/or *B. burgdorferi* s.l. (Table 2), we could not address the effect of ungulates on pathogens in questing ticks in our study area.

Table. 2. Number of questing *Ixodes ricinus* tested for tick-borne pathogens. The number of positive ticks has been given per tick-borne pathogen, including the infection prevalence in brackets and the results of sequencing in grey.

	Nymphs	Adults			Total
		Females	Males	Total	
Number tested	665	35	30	65	730
Anaplasma phagocytophilum	31 [0.05]	2 [0.06]	5 [0.17]	7 [0.11]	38 [0.05]
Ecotype 1	7	1	0	1	8
Ecotype 2	1	0	0	0	1
Unknown ecotype	23	1	5	6	29
Borrelia burgdorferi s.l.	85 [0.13]	5 [0.14]	8 [0.27]	13 [0.20]	98 [0.13]
B. afzelii	20	1	0	1	21
B. burgdorferi s.s.	3	0	0	0	3
B. garinii	8	1	0	1	9
B. valaisiana	3	0	0	0	3
Unknown species	51	3	8	11	62

4.4 Effect of ungulates on small mammals

Through their effect on the vegetation, ungulates might influence the abundance of small mammals. Several studies have indicated that the density of small mammals increases after the exclusion of ungulates (Buesching et al., 2011; Gandy et al., 2021; Keesing, 1998; McCauley et al., 2006). Furthermore, small mammals also play a role in the life cycle of *I. ricinus*, and can thus influence the presence of *I. ricinus*. In our study, we did not catch enough small mammals to explore any associations between ungulate and small mammal densities (Paper III). However, based on the fact that we found an association between the height of the field layer and the passage rates of fallow deer, but not the passage rates of the other ungulate species (Paragraph 4.1), we hypothesize that negative effects of fallow deer on small mammals should be stronger than possible negative effects of the other ungulate species.

4.5 Conclusion

In the study in Paper III, I confirmed the hypothesized effect of ungulates on field layer vegetation, and found that this effect is stronger for the most grazing and gregarious species, fallow deer. The sample sizes for *I. ricinus* and small mammals were too low to draw any conclusions regarding the direct and indirect effects of ungulates on the number of (infected) ticks questing in the vegetation.

5. Changing ungulate communities

In the previous chapters, I showed that ungulates play an essential role in the life cycle of *I. ricinus* and in the transmission of at least some tick-borne pathogens (e.g., *A. phagocytophilum*), and that this role differs among ungulate species, possibly due to their differences in morphology and behaviour. In this chapter, I build on this and use the data from the previous chapters to assess in more detail how changes in the presence and density of common European deer species affect the transmission of *A. phagocytophilum* and *B. burgdorferi* s.l.. Specifically, I am looking at two scenarios: 1) the introduction of red deer and 2) co-varying densities of fallow and roe deer.

5.1 The introduction of red deer

Red deer is one of the species that is expanding its range across Europe (Apollonio et al., 2010; Deinet et al., 2013) and has joined vertebrate communities where they did not occur before (e.g., in northern Sweden). This change in vertebrate communities can affect the circulation of tick-borne pathogens. For example, the prevalence of *A. phagocytophilum* in questing *I. ricinus* nymphs has been linked to the density of red deer, and also fallow deer (Takumi et al., 2021). With the data from our systematic literature search and with the same theoretical host assemblage (Chapter 2), we tested in Paper I if and how the role of the ungulates in the transmission of the ecotypes of *A. phagocytophilum* differ among vertebrate communities with and without red deer (Box 4). We could not do this for fallow deer, due to insufficient data on this species in our systematic literature search. Since small mammals also play a role in the life cycle of *I. ricinus* (Chapter 2), and play a role in the transmission of *A. phagocytophilum* (Bown et al., 2009),

we also included small mammals in our analysis (Box 4). Small mammal densities vary over the years due to population cyclicity, with densities in peak years two to three orders of magnitude higher dan in low-phase years (Andreassen et al., 2021). However, this cyclicity has been flattened in the last decades (Cornulier et al., 2013), which could affect the dynamics of tick-borne pathogens since pathogen transmission and prevalence is dependent on host density in many rodent-borne pathogen systems (e.g., Khalil et al., 2019; Stenseth et al., 2006). In our study (Paper I), we found that the presence of red deer had a positive effect on the number and proportion of feeding adults infected with *A. phagocytophilum* ecotype 1 (Figure 11). This positive effect has been shown in previous studies (Rosef et al., 2009; Takumi et al., 2021), although others failed to find such an effect (Bown et al., 2008; Mysterud et al., 2013). We also found in our study that when small mammals occurred at high densities, the number of feeding larvae and nymphs infected with *A. phagocytophilum* ecotypes 1, 2, and 3 were higher than when they occurred at low densities (Figure 11). To my knowledge, no previous field studies have investigated the relationship between the density of small mammals and the density of *I. ricinus* infected with *A. phagocytophilum*. Our results indicate that the densities at which different host species occur in a host community affect the success of maintenance of a pathogen. However, the exact implications for the circulation of *A. phagocytophilum* cannot be determined with our meta-analysis and more long-term field studies are needed.

Box 4. Investigating the effect of the introduction of red deer.

To investigate the potential effect of changing host communities on the transmission of *A. phagocytophilum*, we established four different scenarios for our theoretical host assemblage from Chapter 2. These scenarios differed in their presence of red deer and their densities of small mammals: a low phase-density and a high phase-density that was a 10-fold higher than the densities in Table 1 on page 24. The presence and density of the other species in the theoretical host assemblage were equal in all scenarios. In all these scenarios we calculated the relative importance of the host taxonomic groups in the transmission of *A. phagocytophilum*. (Paper I)

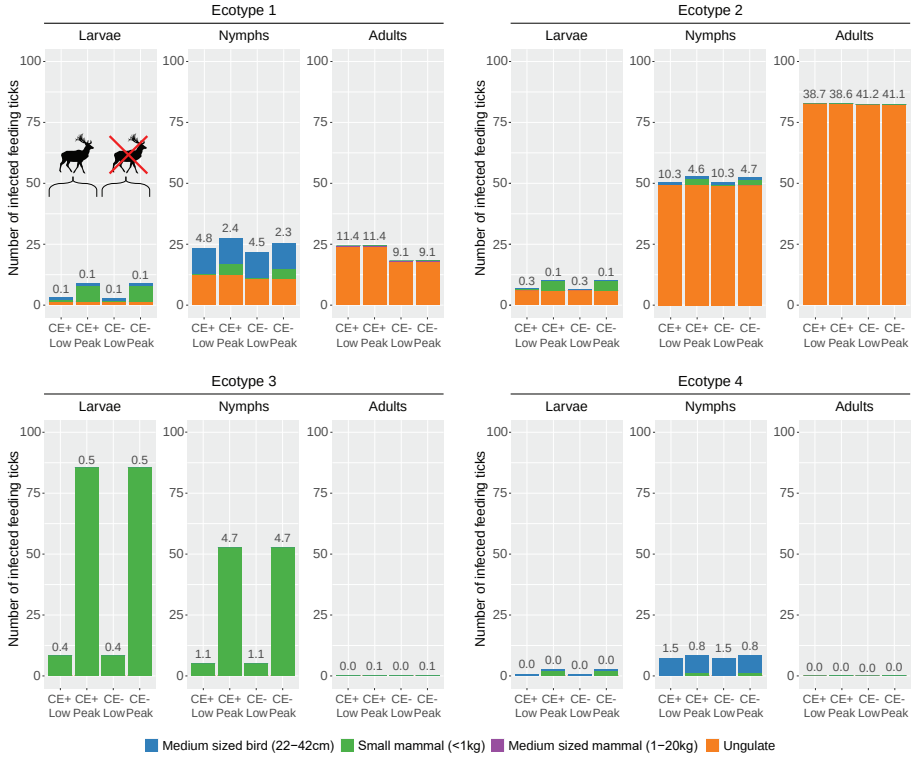

Figure 11. The expected number of feeding *Ixodes ricinus* ticks infected with *Anaplasma phagocytophilum* per life stage, in four host assemblages with varying densities for small mammals and varying presence of red deer. The colours represent the contribution of different host taxonomic groups to the production of these engorged and infected *I. ricinus*. Values on top of bars represent the percentage of feeding ticks that are infected within a host assemblage. CE+ denotes host assemblages with red deer (*Cervus elaphus*) included, while CE- denotes host assemblages where red deer are excluded. In host assemblages denoted with Low, small mammal species were modelled at low phase densities, while in host assemblages denoted with Peak, they were modelled at peak phase densities. Silhouettes by Sander Vink.

5.2 Covarying densities of fallow and roe deer

Wildlife management can influence the composition of ungulate communities. Through management actions, the overall density of ungulates can change, but also the relative abundance of different ungulate species in a community, especially if these species occupy the same niche (Ferretti &

Mori, 2020). Several studies suggest that two of Europe's most common deer species, fallow and roe deer, frequently engage in negative behavioural interactions in favour of fallow deer (Elofsson et al., 2017; Ferretti, 2011; Ferretti et al., 2011; Focardi et al., 2006). The role of these two species in the transmission cycle of *A. phagocytophilum* is quite different, since roe deer are associated with the non-zoonotic ecotype 2 and fallow deer mainly with the zoonotic ecotype 1 (Jaarsma et al., 2019; Jahfari et al., 2014; Chapter 3). Moreover, their role in the life cycle of *I. ricinus* is also slightly different with fallow deer feeding more immature stages (Chapter 3). Therefore, we investigated in Paper IV how co-varying densities of fallow deer and roe deer affect the risk of emergence of tick-borne pathogens, expressed as the basic reproduction number R_0 (According to Hartemink et al., 2008; Matser et al., 2009), using real-life data from an ungulate management experiment in the Amsterdamse waterleidingduinen (Box 5). We also included small mammals and birds in our models (Box 5), due to their role in the life cycle of *I. ricinus* and the transmission of mainly *B. burgdorferi* s.l. (Chapter 2). Our aim was to explore, as a proof-of-concept and in relative terms, how different host community composition matters for the pathogen emergence risks.

Box 5. Investigating the effect of co-varying densities of fallow and roe deer. We constructed a theoretical host assemblage consisting of potential reservoir hosts for *A. phagocytophilum* ecotype 1, ecotype 2, *B. afzelli* and *B. garinii*. We included fallow deer, roe deer and European small mammal and bird species that commonly coexist with these deer species. We used data on co-varying densities of roe deer and fallow deer from the Amsterdamse waterleidingduinen after the introduction of fallow deer in the mid-1990s (FBE Noord-Holland, 2020). We varied small mammal density with a ten-fold difference, and the bird density with a five-fold difference. To characterize the R_0 we used a 6x6 next-generation matrix with larvae, nymphs, fallow deer, roe deer, small mammals and birds (following the approach of Hartemink et al., 2008). We calculated the elasticities to establish the contribution of each species, as described by Hartemink et al. (2008) and Matser et al. (2009). (Paper IV)

In this study in Paper IV, we saw that the value of R_0 for *A. phagocytophilum* ecotype 1 was higher at high fallow deer densities (Figure 12A). At high densities of fallow deer, their contribution to R_0 was nearly 100%. At lower

densities however, other species groups also contributed slightly to the R_0 (See Figure 4 of Paper IV). This is in line with the hypothesis that ecotype 1 is a more generalized pathogen than ecotype 2, which we also found in our study. The value of R_0 for ecotype 2 was lower at high fallow deer and low roe deer densities, and roe deer contributed most strongly to the R_0 (Figure 12B). Since ecotype 1 is zoonotic and ecotype 2 is non-zoonotic, these different contributions of fallow and roe deer to the R_0 for the different ecotypes are important factors for mitigating human and veterinary health risk. Ungulates are thought to be dead-end hosts for genospecies of the *B. burgdorferi* s.l. complex (Mannelli et al., 2012; Ostfeld, 2011), and also in our study we found no visible contribution of the two deer species to the R_0 of *B. afzelii* and *B. garinii*. Small mammals contributed the most to the value of R_0 for *B. afzelii* (See Figure 6 of Paper IV), which is in line with the fact that small mammals are associated with this genospecies (Hanincova et al., 2003). Birds on the other hand contributed the most to the value of R_0 of *B. garinii* (See Figure 7 of Paper IV), confirming previous work that argued that this genospecies is bird-associated (e.g., Taragel'ová et al., 2008). In accordance with these results, we also found that the R_0 of *B. afzelii* and *B. garinii* were higher at high densities of small mammals and birds, respectively. The contribution to the R_0 for both *B. afzelii* and *B. garinii* of both fallow deer and roe deer was negligible, which would suggest that managing deer populations adds little to managing the public health risk of these pathogens, relative to managing small mammal and bird communities. It should be emphasized, however, that we did not explore a scenario of low overall deer densities, and we also did not take into account any ecological interactions in the host community, besides the co-varying densities of fallow and roe deer. Ecological interactions, such as the interaction between ungulates and small mammals, might influence emergence and pathogen dynamics, as has been shown by Roberts and Heesterbeek (2021). Therefore, we could not draw any definitive conclusions regarding the contribution of deer to the value of R_0 for *B. afzelii* and *B. garinii*.

The model in our study uses several parameters to calculate the value of R_0. However, some of these parameters have not been properly studied before, and we therefore had to estimate them (See Appendix A of Paper IV). However, since we wanted to look at the relative effects on the value of R_0, we think that our general results are robust. It was not our aim to obtain

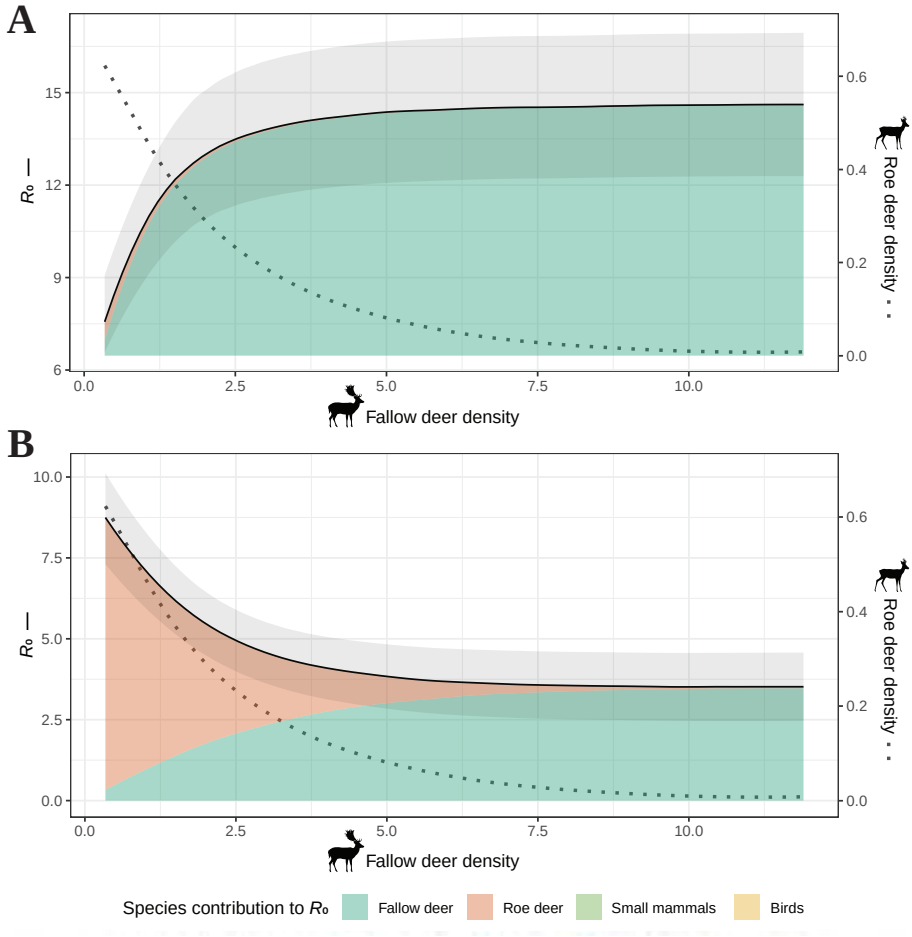

Figure 12. The basic reproduction number R_0 (solid line) of *Anaplasma phagocytophilum* ecotype 1 (A) and ecotype 2 (B) with the standard deviation (grey shading), for co-varying densities of fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) (dotted line) as observed in Amsterdamse waterleidingduinen (FBE Noord-Holland, 2020). The coloured area under the R_0-curve represents the contribution of the different host species groups to the value of R_0. The graphs for each of the scenarios with different small mammal and bird densities are very similar, and therefore only the graph for the scenario with low densities of small mammals and birds is shown. The other graphs can be found in Appendix B of Paper IV.

precise absolute estimates of the R_0 for the pathogens in the different situations. Our study can therefore not be used to determine which management actions can most effectively lower the value of R_0 to below its threshold of 1.

5.3 Conclusion

From the results of both our studies in this chapter, I can conclude that the composition of the ungulate community could affect the establishment of tick-borne pathogens. This effect is probably larger for the establishment of *A. phagocytophilum* than for the establishment of *B. burgdorferi* s.l.. It implicates that ungulate management tailored towards specific ungulate species could be used as a part of zoonotic disease management. However, we only investigated a few tick-borne pathogens, and the outcomes of ungulate management could have a wider range of outcomes for other pathogens and situations. Furthermore, by investigating the differences in R_0 between scenarios, we only investigated the emergence of the pathogens. I cannot say anything how this relates to the risks for humans or the infection prevalence in questing ticks. All this complexity should be taken into account, and I discuss this a bit further in Chapter 6.

6. Ungulate management to mitigate risk

Previous research on the effects of ungulates on ticks and tick-borne pathogens often focused on individual ungulate species (e.g., Michalik et al., 2012; Overzier et al., 2013; Sgroi et al., 2021) or treated multiple ungulate species as one group or black 'ungulate box' (e.g., Dickinson et al., 2020; Gandy et al., 2021; Gilbert et al., 2012; Hofmeester et al., 2017). This ignored the fact that, as I have explained in detail in the previous chapters, these species have different morphological, ecological, and behavioural characteristics that may affect the ability of the ungulate species to feed ticks and to transmit tick-borne pathogens. In this thesis, I tested the main hypothesis that different ungulate species play different roles in the transmission of tick-borne pathogens. I focussed on five common European ungulate species: fallow deer, roe deer, red deer, moose and wild boar, on two tick-borne pathogens: *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l., and on one tick species: *Ixodes ricinus*. In my analyses, I showed that ungulate species differ in how they affect the transmission of tick-borne pathogens, either directly via feeding, and potentially infecting, ticks, or indirectly by shaping the vegetation, which may affect the density of ticks and the density of other potential vertebrate tick hosts. In terms of direct effects, the four deer species played much more significant roles in feeding ticks than wild boar. In terms of indirect effects, I showed that higher fallow deer densities, and not the densities of the other ungulate species, were associated with a lower height of the field layer, but the number of ticks and small mammals recorded was too low for an informative analysis on the link between vegetation and ticks and small mammals (Chapter 4). Overall, however, I found sufficient evidence through my studies that the characteristics of individual ungulate species do indeed matter and that ungulate species can differ strongly in their contribution to tick life cycles

and transmission of tick-borne pathogens. In this chapter, I explore the implications of this finding for ungulate management as a tool to mitigate human and veterinary health risk.

6.1 Implementing ungulate management

Ungulate management could be a potential tool to mitigate human and veterinary health risk of tick-borne pathogens. However, to implement this successfully it is important to know if and how different ungulate species play different roles in the life cycle of ticks and in the transmission of tick-borne pathogens. Based on my results, I can speculate about consequences of ungulate management on tick-borne pathogen transmission. If, for example, the aim is to mitigate the risk of *A. phagocytophilum* for livestock, management actions against roe deer seem to be less effective since they mostly harbour an ecotype that is not pathogenic for livestock (Chapter 3). As another, related, example, during the early 2000s several nature reserves in the Netherlands (e.g., Amsterdamse waterleidingduinen, Deelerwoud), experienced hunting bans that led to strong increases in fallow deer densities and decreases in roe deer densities (FBE Noord-Holland, 2020; Huysentruyt & Casaer, 2015). Based on my results, I would hypothesize that this management action likely increased the human health risk for *A. phagocytophilum* in these areas since fallow deer mainly harbour the zoonotic ecotype 1, while roe deer mainly harbour the non-zoonotic ecotype 2 (Chapter 5). However, we should be careful with drawing conclusions. The exact effects of changes in the host community are even more complex than we show, and we face insufficient data to investigate these full complexities. Furthermore, also other host species in the host community should be taken into account. For example, the removal of an abundantly parasitized host species that is a poor host for a certain pathogen, could increase the infection prevalence in ticks and hosts, if the species that remains is a competent host for the pathogen and ticks (Keesing et al., 2009; 2010).

6.2 Conclusion of the thesis

In this thesis, I confirmed my overall hypothesis that ungulate species identity matters in the role ungulates play in the life cycle of *I. ricinus* and in the transmission of tick-borne pathogens. This indicates that ungulate

management as a tool to mitigate human and veterinary health risk, should be tailored towards the different host species. However, the exact effect of these management actions is complex and depends on the pathogen and target species of the management actions. To avoid unexpected outcomes of management actions to mitigate human and veterinary health risk, more research is needed to finetune how ungulate community composition and dynamics and their ecosystem context shape and influence these risks.

7. Outlook

7.1 Future research directions

While conducting the research for this thesis, I came across specific knowledge gaps and research questions that remain unanswered. These knowledge gaps and questions are important to answer to gain more insight into the role of different ungulate species in the transmission of tick-borne pathogens and into the use of ungulate management to mitigate human and veterinary health risk. In Chapter 2, I came across the fact that many common vertebrate host species are understudied, and especially data on the infection prevalence of *A. phagocytophilum* and *B. burgdorferi* s.l. in feeding ticks is lacking for several vertebrate species that are potentially important for the transmission of these pathogens. For example, fallow deer, a potentially important species for the transmission of *A. phagocytophilum*, and several bird species, like the Eurasian jay and the great spotted woodpecker, potentially important for *B. burgdorferi* s.l.. To get a better overview, these kinds of species need to be included in further research. In Chapter 3, we found differences among the ungulate species in their role in the life cycle of *I. ricinus* and in the transmission of tick-borne pathogens. We did our study, however, at the end of the tick season, and a similar study should be performed more at the peak of the ticks seasons to see if our results still hold. We hypothesize that the differences among ungulate species might even be more pronounced earlier in the season when the density of questing ticks is higher. In Chapter 4, we aimed to study the dynamics between ungulates, the field layer vegetation, small mammal abundance and *I. ricinus* density. Such dynamics are, however, a major challenge to study and one field season is not enough to get robust insight. I expect that a long-term study of perhaps

even a few decades is necessary to gain qualitative and quantitative insights in such dynamics. Our studies in Chapter 2 and 3 were performed in forests in south-central Sweden. Factors like climate, host community densities and habitat type could have influenced our results. It would be good to perform similar studies in areas where these factors differ, to confirm our findings. In Chapter 5, we used fixed densities for the vertebrate hosts in our theoretical host assemblages, except for the co-varying densities of fallow and roe deer. Ecological interactions could, however, influence pathogen dynamics. These interactions include, for example, the interactions between ungulates and small mammals or birds, the interactions of ticks with theirs hosts and the influence of non-host species on the dynamics of host species. These interactions should be taken into account in future research in the ecosystem context. In my thesis, I have focussed on ungulate management, as a tool to mitigate public and veterinary health risk of tick-borne pathogens. However, other management actions could also influence this health risk, like small mammal management and informing people at risk on how to mitigate this risk. Investigation in the impacts of a combination of all these management actions on the circulation of pathogens and public health risk, could give important insights in how to mitigate this risk. Addressing these knowledge gaps, and probably more, is important to be able to 'predict' how specific management actions could affect the circulation of tick-borne pathogens.

7.2 Other ungulate systems

In this thesis, I focused on five ungulate species and on one tick species that are common in Europe. However, the matter of ticks and tick-borne pathogens is not only present in Europe, but world-wide. I therefore asked myself if the conclusion of this thesis, that different ungulate species differ in their role in the transmission of tick-borne pathogens, also holds for other ungulate systems. For example, North America, where the tick species *I. scapularis* is among the most widely distributed ticks (CDC, 2021), hosts a variety of ungulate species. These species include elk (*Cervus canadensis*), white-tailed deer, mule deer (*Odocoileus hemionus*), and Eastern moose (*Alces alces americana*). North-American studies on ungulates and ticks are mainly focused on white-tailed deer, which is considered to be an important host for *I. scapularis* (Ostfeld, 2011). In North America, several variants of *A. phagocytophilum* have been identified, which do not compare with the

ecotypes in Europe (Reviewed in Dugat et al., 2015). White-tailed deer have been found positive with Ap-V1, which is not pathogenic for humans. The variant Ap-ha, which is pathogenic for humans and livestock, has been found in the white-footed mouse (Reviewed in Dugat et al., 2015). It seems thus that the dynamics of the pathogen is different in North America compared to Europe. The North American ungulates are, like the European ones, considered as dead-end hosts for *B. burgdorferi* s.l. (Ostfeld, 2011). With this in mind, and the fact that the North American ungulates also differ in their morphology, ecology, and behaviour, I think the hypothesis that these ungulates differ in their role in the transmission of tick-borne pathogens, also holds in North America. However, at the moment, due to different ungulate behaviour, ungulate morphology and dynamics of pathogens in North America, I cannot say how these roles differ. That can only be determined by a field study where co-occurring ungulate species are investigated for their role in the life cycle of ticks and in the transmission of tick-borne pathogens.

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Popular science summary

In the last couple of decades, different ungulate species (hoofed animals, such as deer and wild boar) have become more abundant and expanded their ranges across large parts of Europe. They are, for example, moving further north into northern Fennoscandia (Denmark, Norway, Sweden and Finland), because warmer winters make it possible for them to live there. Ungulates are important for ticks because ticks can bite them to get a blood meal. Ticks are not only annoying because of their bite, but they can also carry pathogens (for example bacteria and viruses) that they can give to the animals (or persons) they bite. These pathogens can cause diseases, of which Lyme disease is among the best known. However, not all ungulates are the same. For example, a moose is much bigger than a roe deer, and fallow deer live in big herds while a moose lives mostly alone. Also, the species differ in how likely they can become infected by the pathogens carried by the ticks. All these differences might affect how easily a tick can find such an ungulate and attach to it to get a blood meal, and how easily it can infect an ungulate with a pathogen.

In the first part of my thesis, I looked at five different common European ungulate species: fallow deer, roe deer, red deer, moose and wild boar, and investigated if they differed in how many ticks they have. I counted ticks from animals that were shot by hunters and found that there was a big difference between wild boar and the four deer species. Wild boar hardly had any ticks, while the deer species had a lot more ticks. Fallow deer had the most. I also tested if the ticks and the ungulates had any pathogens. Here, I focussed on two different bacteria: *Borrelia burgdorferi*, which causes Lyme disease, and *Anaplasma phagocytophilum*, which causes tick-borne fever in livestock. I saw that ungulates are not very important for the spread of

Borrelia. Wild boar was not very important for the spread of *Anaplasma* either, but the four deer species were. Importantly, there are four different types of *Anaplasma phagocytophilum*, one of which is also dangerous for humans and livestock. Fallow deer, moose and red deer were important species for this type, while roe deer were more important for another type that is not dangerous for humans and livestock.

I also went into the forest and caught ticks that were in the vegetation. These ticks are waiting for an animal (or person) to come by so that they can cling to them and bite them. I did this at different locations that varied in how many of the five ungulate species were present there. At all these spots I also measured how high the vegetation was, and I caught small mammals (like mice). With this information, I wanted to test if the number of the ungulates influences the number of ticks in the vegetation. This influence can go in two ways: directly or indirectly. The direct way is that ticks can feed on the ungulates, and, after they have finished, fall off into the vegetation. The indirect way is a bit more complicated. Ungulates influence the height of the vegetation, by eating and by trampling it. Ticks do not like a lower vegetation, and lower vegetation due to ungulates would thus mean fewer ticks in the vegetation. Small mammals also do not like a lower vegetation, because then they have less protection from predators, like owls. Small mammals also have ticks, and after these ticks are finished eating, they also fall off into the vegetation. Fewer small mammals due to lower vegetation height created by ungulates, would thus also mean fewer ticks in the vegetation. I indeed found that the vegetation was lower when there were more fallow deer. Unfortunately, I did not find enough ticks and small mammals to test my predicted relationships between ungulate grazing, vegetation height, small mammals and ticks.

In the first part of my thesis, I have thus shown that ungulate species differ in how many ticks they have, and in how important they are for the spread of pathogens. I then looked further into a specific example in an area in the Netherlands named Amsterdamse waterleidingduinen, where fallow deer and roe deer occur together. The roe deer have lived there already for a long time, but the fallow deer only since the mid-1990s. Quite soon after fallow deer started living in the area, a hunting ban on fallow and roe deer started. Since this time, the number of fallow deer has increased strongly, while the

number of roe deer has dramatically decreased. I wanted to see if this change in number of both fallow deer and roe deer is important for the spread of the pathogens *Borrelia* and *Anaplasma* in this area. I made a mathematical model and looked how the spread of the pathogens was different for the different numbers of fallow and roe deer. I saw that there was more spread for the type of *Anaplasma phagocytophilum* that is dangerous for humans and livestock when the densities of fallow deer were high, and the densities of roe deer were low. For the type that is not dangerous for humans and livestock it was the other way around. This gives an indication that specific ungulate management decisions, like the ban on hunting in Amsterdamse waterleidingduinen, can play a role in the spread of pathogens and hence in the risk of infection to humans and livestock. In this model, I did not take many other factors that could potentially influence the circulation of the pathogens into account, and I therefore cannot draw definitive strong conclusions about the spread of *Anaplasma* due to changing numbers of fallow and roe deer. But it does clearly show why it is important not just to look at all ungulates as one group, or as I call it in the title of my thesis ‘the ungulate box’, but to look at the different ungulate species separately if we want to understand and predict risk of infection to humans and livestock.

Populärvetenskaplig sammanfattning

Under de senaste årtiondena har olika klövviltssarter, som hjortdjur och vildsvin, blivit vanligare och utökat sitt utbredningsområde över stora delar av Europa. De flyttar till exempel längre norrut in i Fennoskandien (Danmark, Norge, Sverige och Finland) eftersom varmare vintrar gör det möjligt för dem att bo där. Klövvilt är viktiga för fästingar som biter djuren för att komma åt deras blod. Fästingar är inte bara irriterande utan kan också bära på smittämnen (till exempel bakterier och virus) som de kan föra över till djur eller människor. Dessa smittämnen kan orsaka sjukdomar, där borrelios (borreliainfektion; ibland felaktigt kallat för borrelia) är bland de mest kända. Det är dock stor skillnad mellan de olika klövviltsarterna. Till exempel är en älg mycket större än ett rådjur, och dovhjortar lever i stora flockar medan en älg mest lever ensam. Arterna skiljer sig också åt i hur sannolikt de kan bli infekterade av de smittämnen som bärs av fästingar. Alla dessa skillnader kan påverka hur lätt en fästing kan hitta ett klövvilt och fästa vid det, och hur lätt fästingen kan infektera ett klövvilt med en patogen.

I den första delen av mitt avhandlingsarbete tittade jag på fem vanliga europeiska klövviltsarter: dovhjort, rådjur, kronhjort, älg och vildsvin, och undersökte om det skilde sig åt i hur många fästingar de hade. Jag räknade fästingar på djur som skjutits av jägare och fann att det var stor skillnad mellan vildsvin och de fyra hjortarterna. Vildsvin hade knappt några fästingar, medan hjortarterna hade många. Dovhjort hade mest. Jag testade även om fästingarna och klövviltet bar på några smittämnen. Här tittade jag på två olika bakterier: *Borrelia burgdorferi*, som orsakar borrelios, och *Anaplasma phagocytophilum*, som orsakar fästingfeber hos boskap. Jag fann att klövvilt inte är särskilt viktiga för spridningen av *Borrelia*. Vildsvinen var inte heller särskilt viktiga för spridningen av *Anaplasma*, men det var de fyra

hjortarterna. Det finns fyra olika typer av *Anaplasma phagocytophilum*, varav en är farlig för både människor och boskap. Dovhjort, älg och kronhjort var viktiga arter för denna typ, medan rådjur var viktigare för en annan typ som inte är farlig för vare sig människor eller boskap.

Jag begav mig också ut i skogen och fångade fästingar som fanns i vegetationen. Dessa fästingar väntar på att ett djur (eller en människa) ska passera så att de kan haka sig fast och bita dem. Detta gjorde jag på olika platser med varierande täthet av de fem klövviltsarterna. Jag mätte också hur hög vegetationen var och fångade smådäggdjur (exempelvis möss), som fästingar också använder som värddjur. Med denna information testade jag om antalet klövvilt påverkar antalet fästingar, något som kan ske både direkt och indirekt. Den direkta vägen är att fästingar kan livnära sig på klövviltet, sedan släppa taget och falla ned i vegetationen. Det indirekta sättet är lite mer komplicerat; klövvilt påverkar växters höjd genom att äta och trampa på dem. När klövvilt betar och trampar på vegetationen blir den lägre och fästingar gillar inte lägre vegetation. På så vis blir det färre fästingar i vegetationen. Små däggdjur gillar inte heller en lägre vegetation, för då har de mindre skydd mot rovdjur som exempelvis ugglor. Smådäggdjur har också fästingar och efter att dessa fästingar sugit sig fulla med blod släpper även de taget och faller ned i växtligheten. Färre smådäggdjur på grund av lägre vegetationshöjd skapad av klövvilt skulle därmed också innebära färre fästingar i vegetationen. Jag såg att växtligheten var lägre när det fanns fler dovhjortar. Tyvärr hittade jag inte tillräckligt med fästingar och smådäggdjur för att testa mina teorier kring sambandet mellan klövviltsbete, vegetationshöjd, smådäggdjur och fästingar.

I den första delen av min uppsats visade jag att det är skillnad mellan olika klövviltsarter i hur många fästingar de hade på sig och i hur viktiga de är för spridningen av smittämnen. Jag tittade sedan närmare på ett specifikt exempel i Nederländerna vid namn Amsterdamse waterleidingduinen där dovhjort och rådjur förekommer tillsammans. Rådjuren har levt där länge medan dovhjortarna kom dit först vid mitten av 1990-talet. Ganska snart efter att dovhjortar började vistas i området instiftades jaktförbud på dovhjort och rådjur. Sedan dess har antalet dovhjortar ökat kraftigt samtidigt som antalet rådjur har minskat dramatiskt. Jag ville se om denna förändring i antal av både dovhjort och rådjur är viktig för spridningen av smittämnenna *Borrelia*

och *Anaplasma* i detta område. Jag gjorde en matematisk modell och tittade på hur smittämnennas spridning skillde sig åt för olika antal dovhjort och rådjur. Jag såg att spridningen av *Anaplasma phagocytophilum*, den typ som är farlig för människor och boskap, var högre när antalet dovhjortar var högt och antalet rådjur var lågt. För den typen som inte är farlig för människor och boskap var det tvärtom. Detta ger en indikation på att specifika beslut om förvaltning av klövvilt, som jaktförbudet i Amsterdamse waterleiding-duinen, kan spela en roll för spridning av smittämnen och därmed för smittorisken för människor och boskap. I denna modell tog jag inte hänsyn till de många andra faktorer som potentiellt skulle kunna påverka smittämnennas spridning och jag kan därför inte dra definitiva slutsatser om spridningen av *Anaplasma* endast baserat på skiftande antal av dovhort och rådjur. Studien visar dock tydligt varför det är viktigt att inte bara se på klövvilt som en enda grupp, eller som jag kallar det i titeln på min avhandling ‘the ungulate box’, utan att titta på de olika klövviltssarterna separat om vi vill förstå och förutsäga risken för infektion hos människor och boskap.

Populair-wetenschappelijke samenvatting

In de laatste tientallen jaren, komen verschillende hoefdiersoorten, zoals herten en wilde zwijnen, steeds vaker in grotere aantallen in Europa voor. Ook komen ze steeds op meerdere plekken in Europa voor. Ze zijn bijvoorbeeld meer naar het noorden van Fennoscandinavië (Denemarken, Noorwegen, Zweden en Finland) getrokken, omdat warmere winters het voor hen mogelijk maakten om daar te leven. Hoefdieren zijn belangrijk voor teken, omdat teken deze dieren kunnen bijten om een bloedmaaltijd te krijgen. Teken zijn niet alleen vervelend omdat ze bijten, maar ze kunnen ook ziektekiemen dragen (zoals bacteriën en virussen). Deze ziektekiemen kunnen ze dan aan de dieren (of mensen) overdragen en ziektes veroorzaken. De ziekte van Lyme is één van de meest bekende hiervan. Niet alle hoefdieren zijn hetzelfde. Een eland is bijvoorbeeld een stuk groter dan een ree, en een damhert leeft in grote kuddes terwijl elanden alleen leven. Daarnaast verschillen de dieren ook in hoe makkelijk ze besmet kunnen raken met de ziektekiemen van de teken. De verschillen tussen de hoefdieren beïnvloeden de teek in het vinden van en het bijten in hoefdieren om een bloedmaaltijd te krijgen. En ook hoe makkelijk een teek een hoefdier kan besmetten met een ziektekiem.

In het eerste deel van mijn proefschrift heb ik naar vijf verschillende hoefdieren gekeken die veel voorkomen in Europa. Deze vijf soorten zijn: damherten, reeën, edelherten, elanden en wilde zwijnen. Ik heb onderzocht of deze soorten verschillend zijn in het aantal teken dat ze hebben. Ik heb de teken geteld van dieren die door jagers geschoten waren en ik vond dat er een groot verschil was tussen de wilde zwijnen en de vier hertensoorten. Wilde zwijnen hadden bijna geen teken, terwijl de herten er veel meer hadden. Damherten hadden de meeste teken. Ik heb ook de teken en de

hoefdieren getest om te kijken of ze ziektekiemen hadden. Ik keek naar twee verschillende bacteriën: *Borrelia burgdorferi*, welke de ziekte van Lyme kan veroorzaken, en *Anaplasma phagocytophilum*, welke anaplasmose in vee kan veroorzaken. In mijn onderzoek zag ik dat hoefdieren niet erg belangrijk zijn voor de verspreiding *Borrelia*. De wilde zwijnen waren ook niet erg belangrijk voor de verspreiding van *Anaplasma*, maar de vier hertensoorten wel. Het is belangrijk te zeggen dat er vier verschillende soorten van *Anaplasma phagocytophilum* zijn, waarvan er één gevaarlijk is voor mens en dier. De damherten, elanden en edelherten waren belangrijk voor dit type, terwijl reeën belangrijker waren voor een ander type dat niet gevaarlijk is voor mens en dier.

Ik ben ook het bos in gegaan en heb daar teken uit de bodemvegetatie gevangen. Deze teken wachten hier totdat een dier (of mens) langs komt zodat ze zich kunnen vastgrijpen aan hen en kunnen bijten. Ik heb de teken gevangen op verschillende locaties. De vijf verschillende hoefdiersoorten varieerden in aantallen op deze locaties. Op al deze locaties heb ik ook de hoogte van de bodemvegetatie gemeten en heb ik kleine zoogdieren gevangen, zoals muizen. Met al deze informatie wilde ik testen of het aantal hoefdieren, het aantal teken in de bodemvegetatie beïnvloed. Hoefdieren kunnen op twee manieren het aantal teken in de bodemvegetatie beïnvloeden: direct en indirect. De directe manier is dat teken voeden op de hoefdieren, en als ze klaar zijn vallen ze van de hoefdieren af in de bodemvegetatie. De indirecte manier is wat ingewikkelder. Hoefdieren beïnvloeden de hoogte van de bodemvegetatie door het te eten, en door het te vertrappen. Teken houden niet van een kortere bodemvegetatie. Een kortere bodemvegetatie door hoefdieren kan dus leiden tot minder teken in deze bodemvegetatie. Kleine zoogdieren houden ook niet van een kortere bodemvegetatie, omdat ze dan minder bescherming hebben van roofdieren, zoals uilen. Kleine zoogdieren hebben ook teken. Nadat deze teken klaar zijn met voeden vallen ze ook van die dieren af in de bodemvegetatie. Als er minder kleine zoogdieren zijn doordat hoefdieren de bodemvegetatie korter hebben gemaakt, zijn er dus ook minder teken in de bodemvegetatie. Ik heb inderdaad gevonden dat de bodemvegetatie korter was als er meer damherten in het gebied waren. Helaas heb ik niet genoeg teken en kleine zoogdieren kunnen vangen, om te testen of mijn voorspelde relaties tussen het grazen van de hoefdieren, het aantal kleine zoogdieren en het aantal teken klopt.

In het eerste deel van mijn proefschrift heb ik dus laten zien dat hoefdiersoorten verschillen in hoeveel teken ze hebben, en hoe belangrijk ze zijn voor de verspreiding van verschillende ziektekiemen. Hierna heb ik gekeken naar een specifiek voorbeeld in Nederland: de Amsterdamse waterleidingduinen. Hier leven damherten en reeën samen. De reeën leven hier al een hele tijd, maar de damherten pas sinds het midden van de jaren ’90. Vrij snel nadat de damherten in het gebied waren gekomen, werd het verboden om op de herten te jagen. Sinds dit verbod is het aantal damherten enorm gestegen, terwijl het aantal reeën is gedaald in de Amsterdamse waterleidingduinen. Ik wilde uitvogelen of deze verandering in het aantal damherten en reeën belangrijk was voor de verspreiding van de ziektekiemen *Borrelia* en *Anaplasma* in dit gebied. Ik heb hiervoor een wiskundig model gemaakt, en heb gekeken hoe de verspreiding van de ziektekiemen verschilde bij de verschillende aantallen damherten en reeën. Ik zag dat er meer verspreiding was van het type *Anaplasma phagocytophilum* dat gevaarlijk is voor mens en dier als er meer damherten zijn, en minder reeën. Dit was precies andersom voor het type dat niet gevaarlijk is voor mens en dier. Dit is een aanwijzing dat bepaalde beslissingen in het beheer van hoefdieren, zoals het verbieden van jagen in Amsterdamse waterleiding-duinen, een rol kan spelen in het verspreiden van ziektekiemen en dus ook in het risico voor mens en dier. In mijn wiskundig model, heb ik niet alle zaken die mogelijk invloed kunnen hebben op de verspreiding van ziektekiemen meegenomen. Daarom kan ik geen definitieve conclusies trekken over de verspreiding van *Anaplasma* door de verandering in het aantal damherten en reeën. Maar, het geeft wel duidelijk aan dat het belangrijk is om niet alle hoefdiersoorten als één groep te zien, of een ‘ungulate box’ zoals ik het in de titel van mijn proefschrift noem (ungulate is Engels voor hoefdier). De verschillende hoefdiersoorten moeten los van elkaar worden onderzocht als we het risico op besmetting voor mens en dier met ziektekiemen van teken willen begrijpen en voorspellen.

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List of vertebrate species

Scientific	English	Dutch/Nederlands	Swedish/Svenska
Alces alces	Eurasian moose	Europese eland	Europeisk älg
Alces alces americana	Eastern moose	Amerikaanse eland	Amerikansk älg
Apodemus agrarius	Striped field mouse	Brandmuis	Brandmus
Apodemus flavicollis	Yellow-necked mouse	Grote bosmuis	Större skogsmus
Apodemus sylvaticus	Long-tailed field mouse	Bosmuis	Mindre skogsmus
Apodemus uralensis	Ural field mouse	Kleine bosmuis	Dvärgskogsmus
Bison bonasus	European bison	Wisent	Visent
Callosciurus erythraeus	Pallas's squirrel	Pallas' eekhoorn	Pallasekorre
Canis aureus	Golden jackal	Goudjakhals	Guldschakal
Capreolus capreolus	Roe deer	Ree	Rådjur
Carduelis carduelis	European goldfinch	Putter	Steglits
Carduelis chloris	European greenfinch	Groenling	Grönfink
Carduelis spinus	Eurasian siskin	Sijs	Grönsiska
Cervus elaphus	Red deer	Edelhert	Kronhjort
Cervus canadensis	Elk / Wapiti	Wapiti	Vapiti
Coccothraustes coccothraustes	Hawfinch	Appelvink	Stenknäck
Columba livia domestica	Feral pigeon	Stadsduif	Stadsduva
Corvus frugilegus	Rook	Roek	Råka
Corvus monedula	Western jackdaw	Kauw	Kaja
Cricetus cricetus	European hamster	Hamster	Europeisk hamster
Crocidura suaveolens	Lesser white-toothed shrew	Tuinspitsmuis	Trädgårdsnäbbmus
Cyanistes caeruleus	Eurasian blue tit	Pimpelmees	Blåmes
Dama dama	European fallow deer	Damhert	Dovhjort
Dendrocopos major	Great spotted woodpecker	Grote bonte specht	Större hackspett
Erinaceus europaeus	European hedgehog	Egel	Igelkott
Erinaceus roumanicus	Northern white-breasted hedgehog	Oost-Europese egel	Östlig igelkott

Scientific	English	Dutch/Nederlands	Swedish/Svenska
Erithacus rubecula	European robin	Roodborst	Rödhake
Fringilla coelebs	Common chaffinch	Vink	Bofink
Garrulus glandarius	Eurasian jay	Gaai	Nötskrika
Giraffa camelopardalis	Northern giraffe	Giraffe	Giraff
Glis glis	European edible dormouse	Relmuis	Sjusovare
Lacerta agilis	Sand lizard	Zandhagedis	Sandödla
Lacerta bilineata	Western green lizard	Westelijke smaragdhagedis	Västlig smaragdödla
Lacerta viridis	European green lizard	Oostelijke smaragdhagedis	Smaragdödla
Lepus europaeus	European hare	Haas	Fälthare
Lepus timidus	Mountain hare	Sneeuwhaas	Skogshare
Luscinia megarhynchos	Common nightingale	Nachtegaal	Sydnäktergal
Martes foina	Stone marten	Steenmarter	Stenmård
Martes martes	European pine marten	Boommarter	Mård
Meles meles	European badger	Das	Europeisk grävling
Microtus agrestis	Short-tailed field vole	Aardmuis	Åkersork
Microtus arvalis	Common vole	Veldmuis	Fältsork
Microtus oeconomus	Tundra vole	Noordse woelmuis	Mellansork
Microtus subterraneus	European pine vole	Ondergrondse woelmuis	Kortörad gransork
Mus musculus	House mouse	Huismuis	Husmus
Mus spicilegus	Steppe mouse	Steppemuis	Steppmus
Muscardinus avellanarius	Hazel dormouse	Hazelmuis	Hasselmus
Mustela putorius	European polecat	Bunzing	Iller
Myodes glareolus	Bank vole	Rosse woelmuis	Långsvansad skogssork
Myotis myotis	Greater mouse-eared bat	Vale vleermuis	Större musöra
Odocoileus hemionus	Mule deer	Muildierhert	Svartsvanshjort
Odocoileus virginianus	White-tailed deer	Witstaarthert	Vitsvanshjort
Orcinus orca	Killer whale/Orca	Orka/Zwaardwalvis	Späckhuggare
Oryctolagus cuniculus	European rabbit	Europees konijn	Europeisk kanin
Ovis orientalis	European mouflon	Moeflon	Mufflonfår

Scientific	English	Dutch/Nederlands	Swedish/Svenska
Parus major	Great tit	Koolmees	Talgoxe
Periparus ater	Coal tit	Zwarte mees	Svartmes
Peromyscus leucopus	White-footed mouse	Witvoetmuis	Vitfotad hjortråtta
Phalacrocorax carbo	Great cormorant	Aalscholver	Storskarv
Phasianus colchicus	Common pheasant	Fazant	Fasan
Phylloscopus collybita	Common chiffchaff	Tjiftjaf	Gransångare
Phylloscopus trochilus	Willow warbler	Fitis	Lövsångare
Podarcis muralis	Common wall lizard	Muurhagedis	Murödla
Poecile palustris	Marsh tit	Glanskop	Entita
Procyon lotor	Raccoon	Gewone wasbeer	Tvättbjörn
Prunella modularis	Dunnock	Heggenmus	Järnsparv
Pyrrhula pyrrhula	Eurasian bullfinch	Goudvink	Domherre
Rattus rattus	Black rat	Zwarte rat	Svartråtta
Rupicapra pyrenaica	Pyrenean chamois	Pyreneese gems	Pyreneisk gems
Rupicapra rupicapra	Chamois	Gems	Gems
Sciurus carolinensis	Eastern grey squirrel	Grijze eekhoorn	Östlig gråekorre
Sciurus vulgaris	Eurasian red squirrel	Eekhoorn	Ekorre
Sitta europaea	Eurasian nuthatch	Boomklever	Nötväcka
Sorex araneus	Common shrew	Bosspitsmuis	Vanlig näbbmus
Sorex coronatus	Millet's shrew	Tweekleurige bosspitsmuis	Millets näbbmus
Sorex minutus	Eurasian pygmy shrew	Dwergspitsmuis	Dvärgnäbbmus
Sus scrofa	Wild boar	Wild zwijn	Vildsvin
Sylvia atricapilla	Eurasian blackcap	Zwartkop	Svarthätta
Sylvia communis	Common whitethroat	Grasmus	Törnsångare
Talpa europaea	European mole	Mol	Mullvad
Tamias sibiricus	Siberian chipmunk	Siberische grondeekhoorn	Sibirisk jordekorre
Troglodytes troglodytes	Eurasian wren	Winterkoning	Gärdsmyg
Turdus merula	Common blackbird	Merel	Koltrast
Turdus philomelos	Song thrush	Zanglijster	Taltrast
Ursus arctos	Brown bear	Bruine beer	Brunbjörn
Vulpes vulpes	Red fox	Vos	Rödräv

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Parasites & Vectors

RESEARCH Open Access

Wild ungulate species differ in their contribution to the transmission of *Ixodes ricinus*-borne pathogens

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Abstract

Background: Several ungulate species are feeding and propagation hosts for the tick *Ixodes ricinus* as well as hosts to a wide range of zoonotic pathogens. Here, we focus on *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*), two important pathogens for which ungulates are amplifying and dilution hosts, respectively. Ungulate management is one of the main tools to mitigate human health risks associated with these tick-borne pathogens. Across Europe, different species of ungulates are expanding their ranges and increasing in numbers. It is currently unclear if and how the relative contribution to the life-cycle of *I. ricinus* and the transmission cycles of tick-borne pathogens differ among these species. In this study, we aimed to identify these relative contributions for five European ungulate species.

Methods: We quantified the tick load and collected ticks and spleen samples from hunted fallow deer (*Dama dama*, $n = 131$), moose (*Alces alces*, $n = 15$), red deer (*Cervus elaphus*, $n = 61$), roe deer (*Capreolus capreolus*, $n = 30$) and wild boar (*Sus scrofa*, $n = 87$) in south-central Sweden. We investigated the presence of tick-borne pathogens in ticks and spleen samples using real-time PCR. We determined if ungulate species differed in tick load (prevalence and intensity) and in infection prevalence in their tissue as well as in the ticks feeding on them.

Results: Wild boar hosted fewer adult female ticks than any of the deer species, indicating that deer are more important as propagation hosts. Among the deer species, moose had the lowest number of female ticks, while there was no difference among the other deer species. Given the low number of infected nymphs, the relative contribution of all ungulate species to the transmission of *B. burgdorferi* (*s.l.*) was low. Fallow deer, red deer and roe deer contributed more to the transmission of *A. phagocytophilum* than wild boar.

Conclusions: The ungulate species clearly differed in their role as a propagation host and in the transmission of *B. burgdorferi* and *A. phagocytophilum*. This study provides crucial information for ungulate management as a tool to mitigate zoonotic disease risk and argues for adapting management approaches to the local ungulate species composition and the pathogen(s) of concern.

Keywords: *Anaplasma phagocytophilum*, *Borrelia burgdorferi* (*s.l.*), *Ixodes ricinus*, Ungulate management, Zoonotic disease risk

Background

Wild ungulates are common across Europe, and several ungulate species have increased their densities and expanded their ranges during the last decades [1–3]. These changes can be attributed to improved protection,

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the absence of large carnivores in certain areas, food subsidies due to new agricultural and forestry practices and less severe winters [2, 4, 5]. As a result, many areas in Europe currently host a higher diversity of ungulate species than during the recent past [6]. This increase of wild ungulates has allowed their ectoparasites, such as the tick species *Ixodes ricinus*, to increase in densities and expand their ranges [7], leading to an increase in the prevalence of tick-borne zoonotic pathogens, such as *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*). Ungulates play a central role in the life-cycle of *I. ricinus* as feeding hosts, but most importantly as propagation hosts [8]. However, it is poorly understood if and how ungulate species differ in terms of their relative contribution to the tick life-cycle and to the transmission of tick-borne pathogens. Although several studies have looked at the role of ungulates in tick-borne pathogen transmission, only a few of these studied multiple ungulate species simultaneously (e.g. [9–13]). Furthermore, data on certain common ungulate species, particularly wild boar and fallow deer, are currently still scarce [8].

There are several ways in which an ungulate can contribute to the abundance of infected ticks. Two main pathways are: (i) a tick becomes infected while feeding on an ungulate and (ii) an infected tick feeds on an ungulate (regardless of infection status) and detaches fully engorged and still infected. Ungulates also influence the local abundance of infected ticks through their movement, since they might spread the infected ticks to other areas. Aspects that are relevant for pathogen transmission from an infected ungulate to an uninfected tick include the presence of the pathogen in the ungulate, the reservoir competence of the ungulate and the transmission rate of the pathogen from the ungulate to the tick. Ungulates are considered competent hosts for *A. phagocytophilum*, and all European ungulate species can become infected with the pathogen, as has been shown in several studies (reviewed in [14]). However, it is unclear if these species differ in terms of their role in the transmission of *A. phagocytophilum*. Ungulates are not considered to be competent hosts for *B. burgdorferi* (*s.l.*), and it is therefore unlikely that they can transmit this pathogen to ticks [8]. Indeed, it has been proposed that ungulates can have a negative (borreliacidal) effect on the presence of *B. burgdorferi* (*s.l.*) in ticks [9], although the potential impact of this borreliacidal effect remains unclear.

Common and widespread European ungulate species include roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) [6]; in northern Europe, the moose (*Alces alces*) is also widespread and abundant. These species have different morphological and behavioral traits (Table 1), which may influence the likelihood of an ungulate encountering a tick, a tick attaching to an ungulate or an engorged (and infected) tick detaching. For example, variation in leg length among ungulate species may affect the likelihood of attachment by ticks because leg length influences the distance that an adult tick has to travel to preferred feeding sites, such as the axilla and groin [9, 15], and variation in hair structure and skin thickness will likely influence the potential for ticks to penetrate the skin and find a blood meal. Ungulate feeding behavior may also be important in this context since it will be easier for nymphs and larvae to attach to the ears of species that predominately feed in the field layer, such as fallow deer, than to the ears of species that browse higher up, such as moose [9, 15]. In terms of social behavior, grooming behavior and wallowing influence the ability of a tick to fully complete its blood meal, and herd size may influence the likelihood of encountering a tick.

In this study, performed in south-central Sweden, we collected ticks and spleen samples from five common and sympatric European ungulate species, with the aim to determine tick burdens and the prevalence of *A. phagocytophilum* and *B. burgdorferi* (*s.l.*). Based on variation in the aforementioned traits (Table 1), we hypothesized that the ungulate species would differ in their relative contribution as propagation host as well as their role in the transmission of *A. phagocytophilum* and *B. burgdorferi* (*s.l.*). We also present the infection prevalence of *A. phagocytophilum*, *B. burgdorferi* (*s.l.*), *Borrelia miyamotoi* and *Babesia* spp. for the five ungulate species, the infection prevalence of these pathogens in engorged ticks

Table 1 Several traits of five ungulate species

Trait	Fallow deer	Moose	Red deer	Roe deer	Wild boar	References
Body mass (kg)	57	462	240	23	84	[16]
Home range (km^2)	0.7	71.8	54.8	0.5	1.2	[16]
Diet	Grass, fruits and seeds	Trees, shrubs	Trees, shrubs, forbs, grass	Trees, shrubs, crops	Fruits and seeds, grass, crops	[17]
Social structure	Gregarious (big groups)	Solitary	Gregarious (small groups)	Small family groups	Gregarious (big groups)	

collected from the ungulates and the infection prevalence in questing nymphs and adults.

Methods

Sample collection

We opportunistically collected ticks and spleen samples from ungulates shot by hunters on hunting estates in three counties in south-central Sweden: Södermanland, the southernmost part of Stockholm county and the western part of Östergötland (Additional file 1: Figure S1). We selected these areas as they host the most diverse and abundant ungulate populations in Sweden and fall within Sweden's climatic zone where ticks can be abundant [7, 18, 19]. The local habitat is characterized by forests dominated by Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), birch (*Betula* spp.) and European oak (*Quercus robur*), interspersed with agricultural lands with diverse crops [18]. We sampled a total of 324 ungulates: 131 fallow deer, 15 moose, 61 red deer, 30 roe deer and 87 wild boars during October 2018 and October and November 2019. However, not all individuals were sampled for both spleen and ticks (see Additional file 1: Table S1 for a detailed overview).

Hunters gutted each ungulate almost directly after they shot it and, if possible, gave us a part of the spleen. We sampled spleens from the ungulates (in contrast to, for example, sampling blood) since the spleen was easy to collect by the hunters involved in our study and since spleens allow for the detection of multiple tick-borne pathogens simultaneously [11, 20, 21]. Of the 305 animals from which we collected ticks, 182 were checked immediately after gutting; the other 123 animals were stored in cooling chambers (2–6 °C) after gutting and checked 1–6 days after they were shot. Ticks that fell off during this period were not collected. To correct for this, we included the number of days, from the moment the animals were shot until the moment the animals were checked for ticks, in the statistical analysis. We counted the number of ticks separately for eight different body parts (Fig. 1; adjusted from Kiffner et al. [15]). We used forceps to remove all counted ticks and recorded tick life stage and sex, from which part of the body it was collected and whether it was attached, walking or attached to a female (the latter only for males). Furthermore, we recorded the sex and age of the ungulate, and the estate where the animal was shot. We kept all ticks from the same ungulate individual in two sampling tubes with 70% ethanol (one for feeding and one for non-feeding ticks) and stored these at − 20 °C until analysis in the laboratory. Ticks were morphologically identified to species level using morphological keys as described in [22, 23], and all were determined to be *I. ricinus*. This was confirmed microscopically for approximately 30% ($n = 994$) of the ticks.

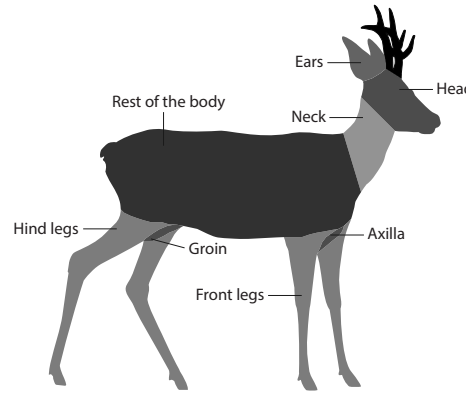

Fig. 1 Illustration of the tick collection sites on roe deer. For the other four ungulate species, we used the same tick collection sites (see Additional file 1: Table S2). Figure is adapted from Kiffner et al. [15]. Silhouette by Sander Vink

In addition to the ticks collected directly from the animals, we also collected questing nymphs ($n = 881$) and adults ($n = 84$) by dragging a 1-m^2 white cotton cloth over the vegetation in the same areas as where the ungulates were shot. The questing ticks also included ticks found on researchers during dragging ($n = 54$). We collected the questing ticks in September 2018 and May–August 2019, similar to the period our sampled ungulates were shot. We counted these questing ticks separately for each life stage and sex (nymphs, males and females) and morphologically identified them to species following [22, 23]. Again, we confirmed our initial morphological determination microscopically for 30% of the individuals ($n = 291$). Of the questing ticks, one male was identified as *Haemaphysalis punctata*, while all others were identified as *I. ricinus.* Sex was not recorded for the adults collected in 2018. We stored the questing ticks individually in 8-strip Eppendorf tubes® (Eppendorf AG, Hamburg, Germany) at − 20 °C.

DNA extraction and pathogen detection

DNA was extracted from unengorged and questing ticks with ammonium hydroxide as described in [24], and DNA was extracted from engorged ticks and spleen samples using the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). We stored the lysates at 4 °C until further analysis. For pathogen detection we used multiplex real-time PCRs on various targeting genes for *A.*

phagocytophilum [25], *B. burgdorferi* (*s.l.*) [26], *B. miyamotoi* [27], *Babesia microti* [28] and *Babesia*-clade X [29]. We followed a qPCR protocol as described in [28]. We amplified all *A. phagocytophilum*-positive spleen samples and 58 of the ticks collected from ungulates that were positive for *A. phagocytophilum* by conventional PCR followed by sequencing to identify an ecotype [25]. Of the ticks collected from ungulates that were positive for *B. burgdorferi* (*s.l.*), we amplified 198 by conventional PCR followed by sequencing to identify the genotype [26]. We did the same for all *Babesia*-positive spleen samples and 64 of the ticks collected from ungulates that were positive for *Babesia* spp. [30]. We could not amplify and sequence all positive ticks due to practical constraints, but previous work has indicated that these sample sizes are representative of the whole population [8]. Furthermore, we did not amplify and type material from any positive questing ticks.

Using body parts as proxy for the whole animal to increase sample sizes

Some of the carcasses were not complete at the moment of tick collection (Additional file 1: Table S1) due to actions by the hunters. We assessed how the number of ticks on certain body parts correlated with the number of ticks found on the whole animal. For this, we included 261 animals for which we checked the complete body for ticks. Of the total number of feeding ticks found on these animals (52 larvae, 1233 nymphs and 966 females), all larvae and > 90% of the nymphs were on the ears, while we found > 90% of the females on the axilla and groin combined (Additional file 1: Table S2). As a result, there was a strong linear correlation between the number of nymphs found on the whole body *versus* on the ears ($R^2_{adj} = 0.999$, $P < 0.001$), and between the number of females found on the whole body *versus* on the axilla and groin combined ($R^2_{adj} = 0.987$, $P < 0.001$). Consequently, in our further analyses, we used the larval and nymphal infestation on the ears as proxies for the total larval and nymphal infestation, respectively, and the female infestation on the groin and axilla combined as a proxy for the total female infestation. This allowed us to increase the sample sizes for our statistical analyses. For the non-feeding male infestation, we only used the ungulate individuals for which we had a full body count, since the males were not attached to their host and therefore not bound to a specific body part.

Contribution of ungulate species as a propagation host

We determined the contribution of each ungulate species as a propagation host by determining the tick burden and the infestation prevalence for female ticks and the infestation prevalence for non-feeding male ticks (depicted by the orange lines in Fig. 2). We calculated the tick burden of female ticks using:

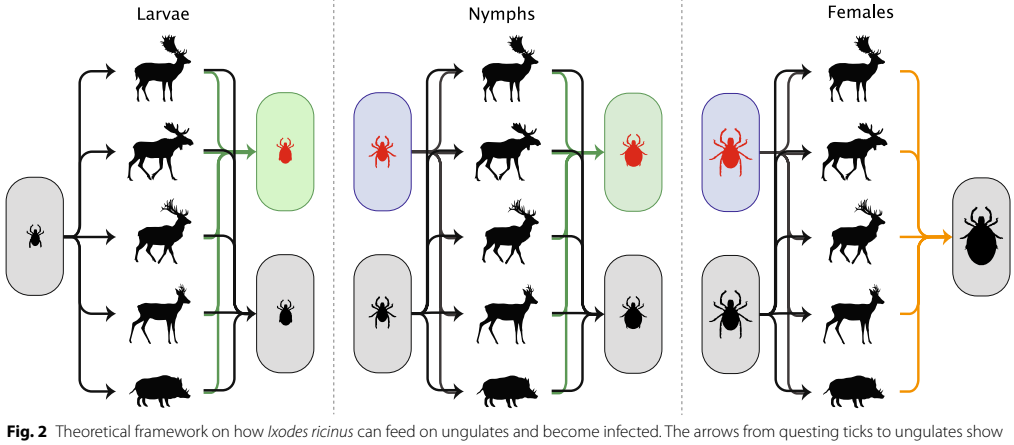

Fig. 2 Theoretical framework on how *Ixodes ricinus* can feed on ungulates and become infected. The arrows from questing ticks to ungulates show the attachment routes, and the arrows from ungulates to engorged ticks show detachment routes. Red ticks are infected ticks, either for *Anaplasma phagocytophilum* or for *Borrelia burgdorferi* (*s.l.*). The engorged females are not divided into infected and uninfected, since we assume that there is no vertical transmission and thus the infection status of an engorged female is irrelevant. The green arrows are the detachment routes of infected larvae and nymphs and show the role of the ungulate species in the transmission of either *A. phagocytophilum* or *B. burgdorferi* (*s.l.*). The orange arrows are the detachment routes of engorged females and show the role of the ungulate species as propagation host. Green boxes show the infection prevalence of engorged ticks, while blue boxes show the infection prevalence in questing ticks. Silhouettes of ungulates by Sander Vink

$$T_{F_i} = P_{F_i} \cdot I_{F_i} \quad (1)$$

where T_{F_i} is the female tick burden on host species i, P_{F_i} is the infestation prevalence of females in host species $i$and I_{F_i} is the infestation intensity of females in host species i. Following Kahl et al. [31] we defined the mean infestation prevalence as the proportion of hosts with feeding ticks on the body parts described above, and the mean infestation intensity as the number of ticks feeding on those body parts, for those hosts that had feeding ticks. For both parameters, we estimated a 95% bootstrapped, bias-corrected, confidence interval (BCa-CI). To calculate the female tick burden, we used the predicted values from the models for the infestation prevalence and intensity of females, which were obtained as described below. To assess differences among the ungulate species in the female tick burden, we compared the 84% bootstrapped, bias-corrected confidence intervals with each other to obtain a significance with an alpha value of 0.05, as suggested by Payton et al. [32].

To obtain predicted values and to test for possible differences among ungulates in the infestation prevalence of females and non-feeding males and the infestation intensity of females, we used hierarchical GLMMs that included, as fixed effects, ungulate species, ungulate sex (female or male), ungulate age group (adult or young) and the number of days between the day the animal was shot and when it was checked for ticks. For the models of infestation prevalence we also included the month of collection (October 2018, October or November 2019) as a fixed effect and a random effect for each hunting estate where the animal was shot (see Additional file 2 for these variables). For the models of infestation intensity, we excluded ungulate species with less than ten individuals infested, since the sample size would be too small. We included a combined random effect for each combination of month of collection and hunting estate due to unbalanced numbers of infested ungulates on the estates over the seasons. We split the hierarchical GLMM by first modeling the infestation prevalence using a GLMM with a binomial distribution. Then, we modeled the infestation intensity, using a GLMM with a zero-truncated negative binomial distribution on a subset of animals on which we found the female tick stage [33]. We fitted GLMMs using the glmmTMB package [34]. We performed model selection, starting with the full models with all above-described parameters as additive effects (i.e. no interactions) using the dredge function in the MuMIn-package. We selected the best fitting models based on the principle of Occam's razor; i.e. from all models with differences in Akaike's information criterion (ΔAIC) < 4, we selected the models with the fewest variables [35].

Contribution of ungulate species to the transmission of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*)

We determined the contribution of a host to the transmission of *A. phagocytophilum* and *B. burgdorferi* (*s.l.*) by quantifying the infection intensity of engorged larvae and nymphs on each ungulate species (depicted by the green lines in Fig. 2). We defined the infection intensity as the mean number of infected ticks found on an individual of each species during the tick-questing period, which is the time when the temperature is above 7 °C, roughly from May until October in our study area [36]. We focused on larvae and nymphs because they molt into infected ticks of the next stage and can ultimately infect another animal or human. We excluded engorged females because they do not produce any infected offspring for either *A. phagocytophilum* or *B. burgdorferi* (*s.l.*) [37–39]. The infection intensity was calculated as:

$$n_{AL_i} = P_{L_i} \cdot I_{L_i} \cdot S_{LA_i} \quad (2)$$

where n_{AL_i} is the *A. phagocytophilum* infection intensity of engorged larvae from host species i, P_{L_i} is the infestation prevalence of larvae from host species i, I_{L_i} is the infestation intensity of larvae from host species i and S_{LA_i} is the *A. phagocytophilum* infection prevalence in larvae from host species i. The *B. burgdorferi* (*s.l.*) infection intensity of engorged larvae (n_{BL_i}), the *A. phagocytophilum* infection intensity of engorged nymphs (n_{AN_i}) and the *B. burgdorferi* (*s.l.*) infection intensity of engorged nymphs (n_{BN_i}) from host species i can be calculated by substituting A by B and/or L by N. We defined the infection prevalence as the proportion of infected ticks among all ticks collected from ungulates, for each tick-borne pathogen, ungulate host species and tick stage. We again estimated the 95% BCa-CIs for all parameters. To calculate the *A. phagocytophilum* and *B. burgdorferi* (*s.l.*) infection intensity of engorged nymphs, we used the predicted values from the models for infestation prevalence and intensity of nymphs and for the *A. phagocytophilum* and *B. burgdorferi* (*s.l.*) infection prevalence, which were obtained as described below. To assess differences among the ungulate species in the infection intensity of engorged larvae and nymphs, we compared the 84% bootstrapped, bias-corrected, confidence intervals with each other to obtain a significance with an alpha value of 0.05 [32].

We performed a Šidák-adjusted Dunn-test to establish if there were any differences in the prevalence of larval infestation, the intensity of larval infestation and the infection prevalence in engorged larvae among the ungulate species. We used this approach because of the small larval sample sizes (Additional file 1: Table S2). To test for possible differences in the infestation prevalence and intensity of nymphs among the ungulate species, we used

hierarchical GLMMs, with the same model structure as described for females and non-feeding males. To test for an effect of ungulate species on the infection prevalence in engorged nymphs, we also used a GLMM with a binomial distribution. We included the same fixed effects as in the GLMM of the infestation intensity, however we excluded the number of days between the day the animal was shot and when it was checked for ticks, since this does not affect the infection status of a tick. We excluded ungulate species with less than ten nymphs tested, since the sample size would be too small. We included a random effect for each host nested within each combination of year of collection (2018 or 2019) and hunting estate.

Pathogen transmission from ungulate host to ticks

To estimate the extent to which ungulate species can infect ticks that feed on them, we compared the infection prevalence of feeding nymphs, feeding females and non-feeding males with the infection prevalence of questing nymphs and questing adults, respectively, with the Šidák-adjusted Dunn-test, for *A. phagocytophilum*, *B. burgdorferi* (*s.l.*), *B. miyamotoi* and *Babesia* spp. We established that there was a difference between the ticks on animals and the questing ticks if the *P*-value was lower than half the alpha value of 0.05.

We performed all analyses in R version 3.6.0 [40] and used an alpha value of 0.05.

Results

The contribution of ungulates as propagation hosts to and the transmission of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*) varied among species (Fig. 3). We describe the results for the different pathways in detail in the following sections.

Contribution of ungulate species as propagation hosts

Of the 261 ungulates of which the whole carcass was checked for ticks, 119 animals were infested with 515 non-feeding males in total (Additional file 1: Table S3). Based on the selected model (Additional file 1: Table S4), moose had the highest infestation prevalence of non-feeding males, and red deer the second highest. Wild boar had the lowest infestation prevalence of non-feeding males, and fallow deer the second lowest, while roe deer did not differ from either red deer or fallow deer (Table 2). We included 300 ungulates that were checked for female ticks on at least both groins and both axillae, where 192 animals were infested with 1179 females in total on the groin and axilla (Additional file 1: Table S3). Based on the selected models (Additional file 1: Table S5), all four deer species had a higher infestation prevalence of females than wild boar, and there was no difference among the deer species in terms of the infestation intensity of females (Table 2). For the model for infestation intensity of females, we excluded wild boar since only five of 82 individuals were infested with females on the groin and axilla (Additional file 1: Table S3). There was no difference in the female tick burden among the deer species (Table 2; Additional file 1: Figure S2), while a female tick burden for wild boar could not be calculated.

Infection prevalence of tick-borne pathogens in ungulates

None of the investigated spleen samples from 64 fallow deer, eight moose, 28 red deer, seven roe deer and 34 wild boars were positive for *B. burgdorferi* (*s.l.*) or *B. miyamotoi*. *Anaplasma phagocytophilum* was found in all ungulate species, although the prevalence was lower in wild boar (Table 3). We determined the ecotype of *A. phagocytophilum* through sequencing of 43 fallow deer, two moose, 20 red deer, seven roe deer and seven wild boars: ecotype 2 was found in all roe deer, while all the other ungulate species harbored ecotype 1. None of the ungulates tested positive for *B. microti*. *Babesia* (*s.s.*) was found in the deer species, but not in wild boar (Table 3). The *Babesia* spp. was determined through sequencing of five fallow deer, three moose, 16 red deer and seven roe deer samples: *B. capreoli* was found in fallow deer and roe deer, *B. divergens* in fallow deer, red deer and roe deer, *B. odocoilei-EU* in fallow deer, moose and red deer and *B. venatorum* in red deer and roe deer (Table 3).

Infection prevalence of tick-borne pathogens in questing ticks

We tested 811 questing *I. ricinus* nymphs and 84 adults for the presence of tick-borne pathogens. All investigated pathogens were present in the questing *I. ricinus* nymphs and adults at low prevalence rates (< 5%), except for *B. burgdorferi* (*s.l.*) and *A. phagocytophilum*, both of which occurred at higher prevalence rates (Table 4). The *Haemaphysalis punctata* male was negative for all investigated pathogens and was excluded from further analyses.

Contribution of ungulate species to the transmission of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*) through larvae

We included 285 ungulates that were checked for larvae and nymphs on at least both ears in the analyses. We found 24 animals that were infested with 59 larvae in total on the ears (Additional file 1: Table S3). A Šidák-adjusted Dunn-test showed that infestation prevalence of larvae differed between fallow deer and red deer ($P = 0.011$) and between fallow deer and wild boar ($P < 0.001$) (Table 2). The infestation intensity of larvae did not differ among the ungulate species (Kruskal–Wallis $\chi^2 = 0.55$; $P = 0.76$) (Table 2).

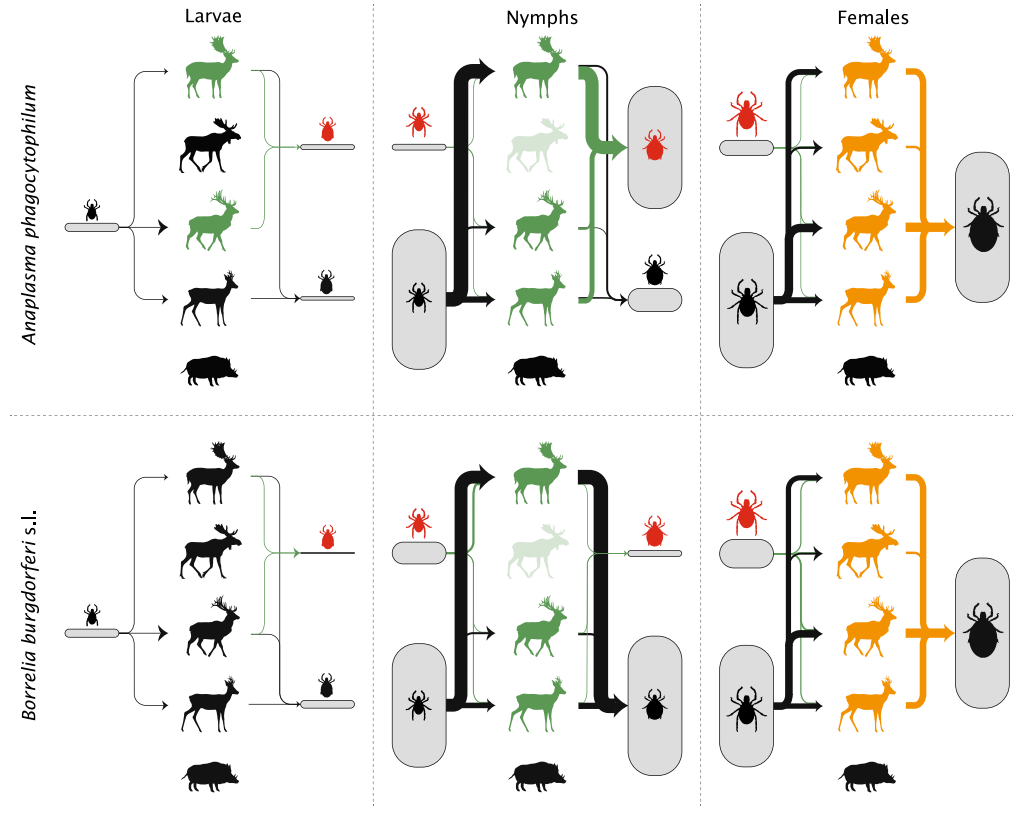

Fig. 3 Illustration of the transmission of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*) by ungulate species. The arrows from questing ticks to ungulates show the attachment routes and the arrows from ungulates to engorged ticks show detachment routes. The thickness of the arrows represents the proportion of ticks attaching or detaching, and the size of the boxes represents the proportion of that tick stage, based on data from Table 2. Red ticks represent infected ticks. The engorged females are not divided into infected and uninfected, since we assume that there is no vertical transmission and thus the infection status of an engorged female is irrelevant. The green arrows and green ungulates show the role of the ungulate species in the transmission of either *A. phagocytophilum* or *B. burgdorferi* (*s.l.*), and the orange arrows and orange ungulates show the role of the ungulate species as propagation host. Light-green coloration of ungulate means that the role of this ungulate in the transmission of either *A. phagocytophilum* or *B. burgdorferi* (*s.l.*) is unknown. Silhouettes of ungulates by Sander Vink

We tested 56 feeding larvae for the presence of tick-borne pathogens. Of these, 77% were positive for *A. phagocytophilum* and 5% for *B. burgdorferi* (*s.l.*) (Additional file 1: Table S6). Sequencing showed that *B. afzelli*, *B. burgdorferi* sensu stricto, *B. garinii* and *B. valaisiana* were present among the ticks (Additional file 1: Table S8). A Šidák-adjusted Dunn-test showed that there was a difference in *A. phagocytophilum* infection prevalence in feeding larvae from red deer and roe deer ($p=0.015$) (Table 2). We found no difference in *B. burgdorferi* (*s.l.*) infection prevalence in feeding larvae among the ungulate species (Kruskal–Wallis test: $\chi^2=2.38$; $P=0.49$) (Table 2).

The *A. phagocytophilum* infection intensity of engorged larvae, calculated with Eq. 2, was the highest for fallow deer and the lowest for moose, roe deer and wild boar, while red deer did not differ from any of the other ungulate species (Table 2; Additional file 1: Figure S3). The *B. burgdorferi* (*s.l.*) infection intensity of engorged larvae did not differ among the ungulate species (Table 2; Additional file 1: Figure S3).

Table 2 Summary of the examined parameters for the five studied ungulate species and tick stage

Parameters	Feeding larvae		Feeding nymphs		Feeding females		Non–feeding males	
Infestation prevalence (95% CI)[a]								
Fallow deer	0.18 (0.11–0.26)	a	0.97 (0.91–0.99)	a	0.95 (0.81–0.99)	a	0.60 (0.40–0.77)	a
Moose	0.00[b]	a,b	0.39 (0.13–0.73)	b,c	0.98 (0.81–1.00)	a	1.00[b]	b
Red deer	0.04 (0.00–0.10)	b	0.66 (0.40–0.85)	b	0.97 (0.88–0.99)	a	0.98 (0.90–0.99)	c
Roe deer	0.10 (0.00–0.21)	a,b	0.94 (0.81–0.99)	a	0.96 (0.78–0.99)	a	0.84 (0.59–0.95)	a,c
Wild boar	0.00[b]	b	0.17 (0.07–0.36)	c	0.03 (0.01–0.17)	b	0.04 (0.01–0.16)	d
Infestation intensity (95% CI)[a]								
Fallow deer	2.47 (1.79–3.84)	a	8.89 (5.80–13.62)	a	3.88 (2.50–6.03)	a		
Moose	0.00		–[d]		1.43 (0.50–4.10)	a		
Red deer	2.50 (2.00–3.00)	a	2.07 (0.54–7.89)	a	5.49 (3.44–8.76)	a		
Roe deer	2.33 (1.00–3.33)	a	3.96 (1.63–9.63)	a	3.93 (2.30–6.71)	a		
Wild boar	0.00		–[d]		–[d]			
Tick burden (95% CI)								
Fallow deer	0.44 (0.17–1.07)	a	8.62 (4.91–14.33)	a	3.67 (1.83–6.37)	a		
Moose	0.00 b		–		1.40 (0.32–4.77)	a		
Red deer	0.10 (0.00–0.30)	b	1.37 (0.17–8.32)	a	5.33 (2.73–9.37)	a		
Roe deer	0.23 (0.00–0.96)	a,b	3.72 (1.12–10.83)	a	3.77 (1.53–7.25)	a		
Wild boar	0.00	b	–		–			
Infection prevalence *Anaplasma phagocytophilum* (95% CI)[a]								
Fallow deer	0.79 (0.62–0.88)	a	0.87 (0.77–0.93)	a				
Moose	0.00[c]	a,b	–[e]					
Red deer	1.00[c]	a	0.87 (0.64–0.96)	a				
Roe deer	0.00[c]	b	0.76 (0.54–0.89)	a				
Wild boar	–		–[e]					
Infection intensity *Anaplasma phagocytophilum* (95% CI)								
Fallow deer	0.35 (0.09–0.98)	a	7.50 (3.48–13.78)	a				
Moose	0.00	b	–					
Red deer	0.10 (0.00–0.36)	a,b	1.19 (0.08–8.95)	a				
Roe deer	0.00	b	2.83 (0.50–10.56)	a				
Wild boar	0.00	b	–					
Infection prevalence *Borrelia burgdorferi* (*s.l.*) (95% CI)[a]								
Fallow deer	0.04 (0.00–0.10)	a	0.04 (0.02–0.06)	a				
Moose	0.00[c]	a	–[e]					
Red deer	0.20 (0.00–0.40)	a	0.04 (0.01–0.12)	a				

Table 2 (continued)

Parameters	Feeding larvae		Feeding nymphs		Feeding females	Non-feeding males
Roe deer	0.00[c]	a	0.04 (0.01–0.09)	a		
Wild boar	–		–[e]			
Infection intensity *Borrelia burgdorferi* (*s.l.*) (95% CI)						
Fallow deer	0.02 (0.00–0.15)	a	0.31 (0.09–0.89)	a		
Moose	0.00	a	–			
Red deer	0.02 (0.00–0.22)	a	0.05 (0.00–1.29)	a		
Roe deer	0.00	a	0.15 (0.01–1.28)	a		
Wild boar	0.00	a	–			

All values for infestation prevalence, infestation intensity and infection prevalence are predicted values from the models in our study, except for the larvae. 95% confidence intervals (CI) are given in parentheses. The lowercase letters indicate the significant differences among the ungulate species

[a] The 95% CI for the infestation prevalence, infestation intensity and infection prevalence are 95% bootstrapped, bias-corrected confidence intervals

[b] The CI for infestation prevalence cannot be calculated if none or all of the animals were infested

[c] The CI for infection prevalence cannot be calculated if none or all of the ticks were infected

[d] The predicted values of the infestation intensity cannot not be obtained due to low number of animals

[e] The predicted values of the infection prevalence cannot be obtained due to low number of tested ticks

Contribution of ungulate species to the transmission of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*) through nymphs

Of the 285 checked individuals, we found 137 animals infested with 1308 nymphs in total on the ears (Additional file 1: Table S3). The selected models (Additional file 1: Table S7) suggested that fallow deer and roe deer had a higher infestation prevalence with nymphs than moose, red deer and wild boar. Of these latter three species, red deer had the highest infestation prevalence with nymphs and wild boar the lowest, while moose did not differ from red deer and wild boar (Table 2). Infestation intensity with nymphs did not differ among fallow deer, red deer and roe deer (Table 2). We excluded moose and wild boar for this parameter because of low sample sizes (Additional file 1: Table S3).

We tested 1309 feeding nymphs for the presence of tick-borne pathogens. Of these, 84% were positive for *A. phagocytophilum* and 5% for *B. burgdorferi* (*s.l.*) (Additional file 1: Table S6). In the models for *A. phagocytophilum* and *B. burgdorferi* (*s.l.*) infection prevalence in nymphs, we excluded moose and wild boar since there were only five and seven nymphs, respectively, tested from these species (Additional file 1: Table S6). Based on the selected models (Additional file 1: Tables S9, S10), there was no difference among fallow deer, red deer and roe deer in terms of the *A. phagocytophilum* and the *B. burgdorferi* (*s.l.*) infection prevalence in nymphs (Table 2).

We could not calculate the infection intensity for moose and wild boar since we did not obtain the infestation intensity due to a low number of animals infested. Among the other ungulate species, we did not find any difference in *A. phatocyophilum* or *B. burgdorferi* (*s.l.*) infection intensity of engorged nymphs (Table 2, Additional file 1: Figure S3).

Transmission of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (*s.l.*) from ungulate host to ticks

The *A. phagocytophilum* infection prevalence was lower in questing nymphs than in feeding nymphs from fallow deer ($P<0.001$), moose ($P=0.005$), red deer ($P<0.001$) and roe deer ($P<0.001$), but not for wild boar ($P=0.038$). The *B. burgdorferi* (*s.l.*) infection prevalence was higher in questing nymphs than in feeding nymphs from fallow deer ($P<0.001$), red deer ($P=0.004$) and roe deer ($P<0.001$), but there was no difference for moose ($P=0.840$) and wild boar ($P=0.703$). We tested 1211 feeding females and 623 non-feeding males derived from ungulates for the presence of tick-borne pathogens and compared the infection prevalence with the infection prevalence in questing adults. Of the feeding females, 92% were positive for *A. phagocytophilum*, as were 76%

Table 3 Infection prevalence of tick-borne pathogens in the five studied ungulate species

Ungulate species	*Anaplasma phagocytophilum*		*Borrelia burgdorferi* (*s.l.*)		*Borrelia miyamotoi*		*Babesia* spp.	
	n_P	IP (95% CI)	n_P	IP (95% CI)	n_P	IP (95% CI)	n_P	IP (95% CI)
Fallow deer ($n=65$)	63[a]	0.98 (0.92–1.00)	0	0.00	0	0.00	9[c]	0.14 (0.06–0.23)
Moose ($n=8$)	8[a]	1.00	0	0.00	0	0.00	5[d]	0.63 (0.13–0.88)
Red deer ($n=28$)	28[a]	1.00	0	0.00	0	0.00	20[e]	0.71 (0.46–0.82)
Roe deer ($n=7$)	7[b]	1.00	0	0.00	0	0.00	7[f]	1.00
Wild boar ($n=34$)	24[a]	0.71 (0.50–0.82)	0	0.00	0	0.00	0	0.00

n_P Number of positive animals, *IP* infection prevalence with 95% CI (95% CI are 95% bootstrapped, bias-corrected CI)

[a] 42 *A. phagocytophilum*-positive samples from fallow deer, two from moose, 20 from red deer and seven from wild boar were sequenced; all were ecotype 1

[b] All *A. phagocytophilum*-positive samples from roe deer were sequenced; all were ecotype 2

[c] Eight *Babesia* spp.-positive samples from fallow deer were sequenced: two *B. capreoli*, two *B. divergens* and three *B. odocoilei-EU*

[d] Three *Babesia* spp.-positive samples from moose were sequencend: *B. odocoilei-EU*

[e] 16 *Babesia* spp. positive-samples from red deer were sequenced: six *B. divergens*, three *B. odocoilei-EU*, one *B. venatorum* and six *B. divergens* and *B. venatorum*

[f] All *Babesia* spp.-positive samples from roe deer were sequenced: three *B. capreoli*, three *B. capreoli* and *B. venatorum* and one *B. capreoli*, *B. divergens* and *B. venatorum*, respectively

of the males (Additional file 1: Table S6). The infection prevalence in questing adults was lower than that in feeding females from all ungulates ($P<0.001$) and it was lower than the infection prevalence in non-feeding males from fallow deer ($P<0.001$), moose ($P<0.001$), red deer ($P<0.001$) and roe deer ($P<0.001$). There was no difference in infection prevalence between the questing adults and the non-feeding males from wild boar. We found that 8% of the feeding females and 14% of the non-feeding males were positive for *B. burgdorferi* (*s.l.*) (Additional file 1: Table S6). The infection prevalence was higher in questing adults than in feeding females from fallow deer ($P=0.023$), moose ($P=0.024$), red deer ($P<0.001$) and roe deer ($P<0.001$), while there was no difference for wild boar ($P=0.807$). For all ungulate species there was no difference in infection prevalence between the non-feeding males and questing adults (Kruskal–Wallis test: $\chi^2=6.75$; $P=0.24$).

Babesia spp. and *Borrelia miyamotoi* in ticks collected from ungulates

Of the feeding larvae, 4% were positive for *Babesia* spp., as were 5% of the feeding nymphs, 13% of the feeding females and 6% of the non-feeding males (Additional file 1: Table S6). Among the positive ticks, we found *B. microti*, *B. capreoli*, *B. venatorum*, *B. divergens* and *B. odocoilei-EU* (Additional file 1: Table S11). The infection prevalence of *Babesia* spp. was higher in feeding nymphs than in questing nymphs for red deer ($P<0.001$) and roe deer ($P<0.001$), while there was no difference for the other ungulate species. The infection prevalence was higher in feeding females than in questing adults for red deer ($P<0.001$) and roe deer ($P=0.009$), but not for the other ungulate species. There was no difference in infection prevalence between the non-feeding males and questing adults for any of the ungulate species (Kruskal–Wallis test: $\chi^2=7.30$; $P=0.20$) .

Furthermore, we found that 2% of feeding larvae, 2% of feeding nymphs, 1% of feeding females and 0.5% of non-feeding males were positive for *B. miyamotoi* (Additional file 1: Table S6). The infection prevalence of feeding nymphs, feeding females and non-feeding males was not different from the infection prevalence of questing nymphs and questing adults, respectively (all Kruskal–Wallis test: Nymphs, $\chi^2=5.03$; $P=0.41$; Females, $\chi^2=9.11$; $P=0.10$; Males, $\chi^2=3.78$; P value $=0.58$).

Discussion

In this study we determined the relative contribution of different ungulate species as propagation hosts by comparing the infestation prevalence of non-feeding males and the female tick burden. All deer species we studied had a similar female tick burden and infestation prevalence of non-feeding males and, thus, played a similar role as propagation host in the life-cycle of *I. ricinus* (Fig. 3). For wild boar, we could not calculate the female tick burden due to a low number of *I. ricinus*-infested individuals despite a relatively high number of sampled individuals. Based on this low number, and on the low infestation prevalence, we conclude that, in our study, the role of wild boar as propagation host is negligible (Fig. 3). The contribution of the ungulate species to the transmission of *A. phagocytophilum* and *B. burgdorferi* (*s.l.*) was determined by comparing the infection intensity in larvae and nymphs. The *A. phagocytophilum* infection intensity in larvae was higher in fallow deer and red deer than in the other studied ungulate species. In nymphs, it was similar for fallow deer, red deer and roe deer, but

Table 4 Infection prevalence of tick-borne pathogens in questing *Ixodes ricinus* ticks

Life stage	*Anaplasma phagocytophilum*		*Borrelia burgdorferi* (*s.l.*)		*Borrelia miyamotoi*		*Babesia* spp.	
	n_P	IP (95% CI)	n_P	IP (95% CI)	n_P	IP (95% CI)	n_P	IP (95% CI)
Nymphs ($n=881$)	36	0.04 (0.03–0.05)	136	0.15 (0.13–0.18)	8	0.01 (0.00–0.02)	8	0.01 (0.00–0.02)
Adults ($n=84$)	8	0.10 (0.04–0.15)	16	0.19 (0.11–0.26)	1	0.01 (0.00–0.04)	3	0.04 (0.00–0.07)

n_P and IP (95% CI) are as defined in footnote of Table 3

could not be determined for wild boar and moose due to the low number of individuals infested with nymphs. Due to the low infestation prevalence in wild boar, we conclude that the role of wild boar in the transmission of *A. phagocytophilum* is negligible compared to that of fallow deer, red deer and roe deer (Fig. 3). For moose, we cannot draw definitive conclusions since the number of sampled moose was too low. The *B. burgdorferi* (*s.l.*) infection intensity in larvae was similar among all studied ungulate species, as well as in nymphs for fallow deer, red deer and roe deer. Again, we could not determine the *B. burgdorferi* (*s.l.*) infection intensity for wild boar and moose. However, given the low prevalence rates we can conclude that the role of wild boar in *B. burgdorferi* transmission is negligible compared to fallow deer, red deer and roe deer (Fig. 3).

The infestation prevalence and intensity varied among the ungulate species in our study, but in general we found lower numbers than other studies previously conducted in Europe [8–10, 41, 42]. Since the questing tick densities in our area were also lower than in other European studies, we believe that the main reason for the lower infestation prevalence and intensity might be geographical. Aspects like climate, vegetation and general mammal density might be different in our study area than elsewhere in Europe and explain lower tick densities. However, another reason might be that we sampled ticks from hunted animals and were therefore restricted to the hunting season to obtain abundant ungulate samples. Hunting season, however, occurs towards the end of the tick season, while in other studies sampling occurred either during the peak season or throughout the season. The fact that we sampled late in the season could therefore also partly explain the relatively low infestation prevalence and intensity we found. For all ungulate species, we found a low larval tick burden, which has also been found in other studies in Europe [43–46]. We found the majority of nymphs attached to the ears of ungulates, while adults were mainly attached to the groin and axilla, which is in line with results of previous studies on roe deer [9, 15]. The aim of our study was to test if and how ungulate species identity matters in terms of the spread of tick-borne pathogens—and not to determine the absolute tick burden and infection prevalence of the ungulate species. We conclude that ungulate species does indeed matter for this part of Sweden and during the late season. This is an important finding and highlights that we should investigate whether the differences among ungulate species that we found hold for other areas in Europe and/or during other seasons. For example, the differences among species that we identified may be even more pronounced earlier in the season, when the density of questing ticks is higher.

Our findings provide initial support for our suggestion that behavioral and morphological traits might drive differences in the role of different ungulate species in the life-cycle of *I. ricinus*. The concentration of adult ticks in the groin and axilla of all species indicates that, although the access points for ticks on different ungulate species might differ [47], most adult ticks migrate to the groin and axilla, suggesting that host leg length could play a larger role in determining the tick burden of ungulates than body mass. This may explain why we found a relatively low tick burden on moose, which have particularly long legs [48]. Moreover, we found the highest infestation prevalence and intensity of nymphs on the ears of fallow deer, which supports our hypothesis that feeding type influences tick infestation rates, since fallow deer graze more than the other deer species [17]. Ticks, which are mostly questing on ground vegetation, will more easily access grazing ungulates *via* the ears (and the head) than species that more frequently browse vegetation strata higher up, such as moose [49].

For all ungulate species, the *A. phagocytophilum* infection prevalence we found in feeding ticks (0.76–1.00) was high relative to values reported in earlier studies (0.22–0.86) [11, 43, 50]. Furthermore, we found a higher infection prevalence in feeding ticks than in questing ticks. This suggests that ungulates are important transmission hosts for *A. phagocytophilum* in this part of Sweden, despite tick infestation being relatively low, and that the infection prevalence of ungulate hosts influences the infection prevalence in feeding ticks. Within Europe there is much variation in infection prevalence in ungulate hosts [14], which might explain why the infection prevalence in feeding ticks reported in other studies was lower. In our study, the transmission cycle of *A. phagocytophilum* was mainly between nymphs and females.

This has been proposed [51], but had not been shown in a field study. Moreover, non-feeding males in our study were infected with *A. phagocytophilum* and this infection prevalence was higher than in questing adults. This finding may suggest that *A. phagocytophilum* alters tick behavior, causing them to select for ungulates, or that males actually become infected with *A. phagocytophilum* between the time of questing and when we collected them from the animals [52]. The latter might happen when a male briefly feeds on a host before finding a female to mate with, or males might feed on the females they are attached to during mating and become infected through the female. However, our data do not allow us to draw conclusions on exact transmission pathways and we encourage others to investigate these potential mechanisms in targeted studies. More generally, our interpretation of the differences in infection prevalence between questing ticks and feeding ticks has its limitations because we did not investigate the exact transmission dynamics of tick-borne pathogens. However, our results can be used to generate hypotheses on the role of different ungulate species in the transmission pathways of tick-borne pathogens, which should be further investigated in future research. In fact, this remains a major knowledge gap for the field of tick-borne pathogens in general.

For *B. burgdorferi* (*s.l.*) we found a low infection prevalence in feeding ticks for all ungulate species, reflecting results in other studies [9, 10, 41, 42, 53, 54]. This low infection prevalence in feeding ticks for *B. burgdorferi* (*s.l.*), combined with our finding that infection prevalence was lower in feeding ticks than in questing ticks, support the notion that ungulates do not transmit the bacterium and that there might even be a borreliacidal effect [9, 55]. However, we still found *B. burgdorferi* (*s.l.*) in engorged ticks, which has also been shown in other studies [9, 10, 41, 42, 54]. Although these observations contradict the borreliacidal effect, we cannot rule out that the *B. burgdorferi* (*s.l.*) we detected in ticks were not-infectious bacteria. The infection prevalence in feeding larvae was low but not zero, which might indicate some co-feeding transmission between feeding nymphs or females and feeding larvae. Co-feeding transmission of *B. burgdorferi* (*s.l.*) has not yet been identified in ungulates, but has been demonstrated in mice (reviewed in [56]).

Although we made every effort to collect a sufficient sample size, the sample size for several of our ungulate species was still quite limited (especially for moose and roe deer). This was, at least partly, due to the relatively low densities of these species in our study area [57] and the resulting low hunting quota. These low sample sizes might explain why some of the differences among the ungulate species in terms of female tick burden and infection intensity in larvae and nymphs were non-significant. The aim of our study was to compare different ungulate species and, therefore, we only draw conclusions on the relative contribution of the five studied ungulate species. To investigate the overall contribution of ungulates species, we should have included other host species in our study. Furthermore, we cannot draw any conclusions about the absolute tick burden and infection prevalence for each ungulate species. Our initial results, which indicate that ungulate species identity matters, do strongly suggest that future research should quantify the absolute contribution of different ungulate species to the dynamics of tick-borne pathogens. Such work should focus on essential parameters, such as exact transmission pathways and persistent infection, which we did not include in our study. Such studies have been performed in rodents (e.g., [28]), but not yet in ungulates.

Our study included only *I. ricinus* and included tick-borne pathogens for which this tick species is the main vector in Europe [58]. However, we do suggest that similar results may hold for a broader collection of tick species and their pathogens. The main pathogens investigated in our study, *B. burgdorferi* (*s.l.*) and *A. phagocytophilum*, are globally not limited to the tick species *I. ricinus* [58], and it is likely that the morphological and behavioral differences among the ungulate species also influence their ability to feed other tick species.

Conclusion

Despite our relatively low sample sizes, we found support for our main hypothesis that the different ungulate species may play a different role in the propagation of ticks and the transmission cycles of tick-borne pathogens. For example, in our system wild boar played a small role as propagation host, and fallow deer seemed to play a stronger role in the transmission cycle of *A. phagocytophilum* relative to the other deer species. Given our small sample sizes, we urge others to challenge and confirm our preliminary findings and invest more effort in comparing the role of different sympatric ungulate species in the spread of tick-borne pathogens in other systems and during other seasons. If our results hold, this means that ungulate management, as a tool to mitigate zoonotic disease risk, should not treat ungulates as one black box. Rather, such management should take the potentially different roles of different species as propagation hosts and in pathogen transmission into account and acknowledge that these roles may vary depending on the target pathogen. Our initial results suggest that choices in ungulate management, for example targeting specific ungulate species differently, could markedly influence the impact of the strategy on the abundance of infected questing ticks.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-021-04860-w.

Additional file 1: Table S1. Number of individuals per ungulate species included in the study. **Table S2.** Proportion (%) of ticks found on different body parts of the five studied ungulate species. **Table S3.** Summary of feeding larvae and feeding nymphs on ears, feeding females on groin and axilla and non-feeding males on complete carcasses on five ungulate species. **Table S4.** Standardized model estimates with 95% confidence intervals for the analysis of infestation prevalence with non-feeding males. **Table S5.** Standardized model estimates with 95% confidence intervals for the analysis of infestation prevalence (A) and intensity (B) with feeding females. **Table S6.** Infection prevalence of tick-borne pathogens in feeding *Ixodes ricinus* ticks from five studied ungulate species. **Table S7.** Standardized model estimates with 95% confidence intervals for the analysis of infestation prevalence (A) and intensity (B) with feeding nymphs. **Table S8.** Sequencing results from *Borrelia burgdorferi* (*s.l.*) positive ticks collected from ungulates. **Table S9.** Standardized model estimates with 95% confidence intervals for the analysis of the infection prevalence of *Anaplasma phagocytophilum* in feeding nymphs. **Table S10.** Standardized model estimates with 95% confidence intervals for the analysis of the infection prevalence of *Borrelia burgdorferi* (*s.l.*) in feeding nymphs. **Table S11.** Sequencing results from *Babesia* ssp. positive ticks collected from ungulates. **Figure S1.** Map of Sweden with the study area in green. **Figure S2.** Larval (**A**), nymphal (**B**) and female (**C**) tick burden on the studied ungulate species. Tick burden, as calculated by formula 1, is given with 84% bootstrapped, bias-corrected, confidence intervals to show differences among ungulate species with a significance with an alpha value of 0.05. **Figure S3.** Infection intensity in larvae and nymphs from the studied ungulate species. Infection intensity, as calculated by formula 2, is given with 84% bootstrapped, bias-corrected, confidence intervals to show differences among ungulate species with a significance with an alpha value of 0.05. The four graphs show the *Anaplasma phagocytophilum* infection intensity in larvae (**A**) and nymphs (**B**) and the *Borrelia burgdorferi* (*s.l.*) infection intensity in larvae (**C**) and nymphs (**D**).

Additional file 2: Raw dataset.

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Authors' contributions
NF, JC, HS and FW designed the methodology. NF and BD collected the field data. HS, NF and BD performed the lab analyses. NF and TH analyzed the data. NF, JC and HS led the writing of the manuscript. All authors contributed critically to the drafts, and all authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its Additional files.

Declarations

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Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

Wild ungulate species differ in their contribution to the transmission of Ixodes ricinus-borne pathogens.

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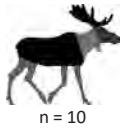
Additional file 1: Additional tables and figures

Table S1. Number of individuals per ungulate species included in the study.

	Fallow deer	Moose	Red deer	Roe deer	Wild boar
Individuals where a part of the carcass was checked for ticks and no spleen sample was obtained	15	4	6	3	1
Individuals where the whole carcass was checked for ticks and no spleen sample was obtained	52	3	27	20	52
Individuals where only a spleen sample was obtained	16	0	2	0	1
Individuals where a part of the carcass was checked for ticks and a spleen sample was obtained	7	1	3	1	3
Individuals where the whole carcass was checked for ticks and a spleen sample was obtained	41	7	23	6	30
Total number of individuals	131	15	61	30	87

Table S2. Proportion (%) of ticks found on different body parts of the five studied ungulate species.

	Feeding larvae		Feeding nymphs		Feeding females	
Fallow deer	Larvae (n=42)		Nymphs (n=1015)		Females (n=464)	
	**Ears**	**97.6**	**Ears**	**98.8**	Ears	0.7
	Head	0	Head	0.1	Head	0.2
	Neck	0	Neck	0	Neck	0
	Front leg	0	Front leg	0	Front leg	0
	Axilla	0	Axilla	0.6	Axilla	8.2
	Hind leg	0	Hind leg	0	Hind leg	0.2
n = 93	Groin	0	Groin	0.2	**Groin**	**88.1**
infested = 88	Other	0	Other	0	Other	1.1
	Unknown	2.4	Unknown	0.2	Unknown	1.5
Moose	Larvae (n=0)		Nymphs (n=2)		Females (n=26)	
			**Ears**	**100**	Ears	15.4
			Head	0	Head	0
			Neck	0	Neck	3.8
			Front leg	0	Front leg	0
			Axilla	0	Axilla	15.4
			Hind leg	0	Hind leg	0
n = 10			Groin	0	**Groin**	**65.4**
infested = 10			Other	0	Other	0
			Unknown	0	Unknown	0
Red deer	Larvae (n=3)		Nymphs (n=88)		Females (n=331)	
	**Ears**	**100**	**Ears**	**97.8**	Ears	1.5
	Head	0	Head	1.1	Head	0
	Neck	0	Neck	0	Neck	0.3
	Front leg	0	Front leg	0	Front leg	0
	Axilla	0	Axilla	0	Axilla	7.6
	Hind leg	0	Hind leg	0	Hind leg	0
n = 50	Groin	0	Groin	0	**Groin**	**89.1**
infested = 47	Other	0	Other	0	Other	0.9
	Unknown	0	Unknown	1.1	Unknown	0.6
Roe deer	Larvae (n=7)		Nymphs (n=122)		Females (n=132)	
	**Ears**	**100**	**Ears**	**99.2**	Ears	0.8
	Head	0	Head	0	Head	0
	Neck	0	Neck	0	Neck	0
	Front leg	0	Front leg	0	Front leg	0
	Axilla	0	Axilla	0	Axilla	11.4
	Hind leg	0	Hind leg	0.8	Hind leg	2.3
n = 26	Groin	0	Groin	0	**Groin**	**85.6**
infested = 24	Other	0	Other	0	Other	0
	Unknown	0	Unknown	0	Unknown	0
Wild boar	Larvae (n=0)		Nymphs (n=6)		Females (n=13)	
			**Ears**	**100**	Ears	7.7
			Head	0	Head	0
			Neck	0	Neck	0
			Front leg	0	Front leg	0
			Axilla	0	Axilla	7.7
			Hind leg	0	Hind leg	0
n = 82			Groin	0	**Groin**	**53.8**
infested = 13			Other	0	Other	30.8
			Unknown	0	Unknown	0

Percentages higher than 50% are given in bold and number of samples are given in parentheses. Only individuals of which the entire carcass was checked for ticks were included. Silhouettes by Sander Vink.

Table S3. Summary of feeding larvae and feeding nymphs on ears, feeding females on groin and axilla and non-feeding males on complete carcasses on five ungulate species.

	Ears			*Groin and axilla*		*Complete carcass*	
	n_e	**Feeding larvae**	**Feeding nymphs**	n_{ga}	**Feeding females**	n_c	**Non-feeding males**
Fallow deer	104	Larvae (n=47) Infested individuals = 19 Mean D_L = 0.45 (0.25-0.84) Mean P_L = 0.18 (0.11-0.26) Mean I_L = 2.47 (1.79-3.84)	Nymphs (n=1 071) Infested individuals = 88 Mean D_N = 10.30 (8.16-13.71) Mean P_N = 0.85 (0.76-0.90) Mean I_N = 12.17 (10.13-16.57)	115	Females (n=514) Infested individuals = 99 Mean D_F = 4.47 (3.70-5.36) Mean P_F = 0.86 (0.77-0.91) Mean I_F = 5.19 (4.47-6.19)	93	Males (n=108) Infested individuals = 44 Mean D_M = 1.16 (0.77-1.87) Mean P_M = 0.47 (0.37-0.57) Mean I_M = 2.45 (1.80-3.94)
Moose	15	Larvae (n=0) Infested individuals = 0 Mean D_L = 0.00 Mean P_L = 0.00 Mean I_L = 0.00	Nymphs (n=4) Infested individuals = 3 Mean D_N = 0.27 (0.00-0.60) Mean P_N = 0.20 (0.00-0.40) Mean I_N = 1.33 (1.00-1.67)	14	Females (n=33) Infested individuals = 13 Mean D_F = 2.36 (1.44-3.57) Mean P_F = 0.93 (0.64-1.00) Mean I_F = 2.54 (1.62-4.01)	10	Males (n=66) Infested individuals = 10 Mean D_M = 6.60 (4.20-8.80) Mean P_M = 1.00[b] Mean I_M = 6.60 (4.14-8.90)
Red deer	52	Larvae (n=5) Infested individuals = 2 Mean D_L = 0.10 (0.00-0.29) Mean P_L = 0.04 (0.00-0.10) Mean I_L = 2.50 (2.00-2.50)	Nymphs (n=97) Infested individuals = 18 Mean D_N = 1.87 (0.84-3.68) Mean P_N = 0.35 (0.21-0.46) Mean I_N = 5.39 (2.72-10.04)	58	Females (n=384) Infested individuals = 50 Mean D_F = 6.62 (5.17-8.61) Mean P_F = 0.86 (0.71-0.91) Mean I_F = 7.68 (6.00-9.82)	50	Males (n=269) Infested individuals = 44 Mean D_M = 5.38 (3.92-7.29) Mean P_M = 0.88 (0.76-0.94) Mean I_M = 6.11 (4.59-8.16)
Roe deer	29	Larvae (n=7) Infested individuals = 3 Mean D_L = 0.24 (0.03-0.72) Mean P_L = 0.10 (0.00-0.21) Mean I_L = 2.33 (1.00-3.33)	Nymphs (n=129) Infested individuals = 21 Mean D_N = 4.45 (2.90-7.39) Mean P_N = 0.72 (0.52-0.83) Mean I_N = 6.14 (4.03-9.80)	29	Females (n=133) Infested individuals = 25 Mean D_F = 4.59 (3.28-6.31) Mean P_F = 0.86 (0.62-0.93) Mean I_F = 5.32 (4.03-6.88)	26	Males (n=68) Infested individuals = 19 Mean D_M = 2.62 (1.75-3.76) Mean P_M = 0.73 (0.42-0.85) Mean I_M = 3.58 (2.68-4.79)
Wild boar	85	Larvae (n=0) Infested individuals = 0 Mean D_L = 0.00 Mean P_L = 0.00 Mean I_L = 0.00	Nymphs (n=7) Infested individuals = 7 Mean D_N = 0.08 (0.02-0.14) Mean P_N = 0.08 (0.02-0.14) Mean I_N = 1.00[a]	86	Females (n=8) Infested individuals = 5 Mean D_F = 0.09 (0.03-0.22) Mean P_F = 0.06 (0.01-0.12) Mean I_F = 1.60 (1.00-2.20)	82	Males (n=4) Infested individuals = 2 Mean D_M = 0.05 (0.00-0.20) Mean P_M = 0.02 (0.00-0.06) Mean I_M = 2.00 (1.00-3.00)

n_e = number of animals where at least the ears were checked for ticks, n_{ga} = number of animals where at least the groin and axilla were checked for ticks, n_c = number of animals where the complete carcass was checked for ticks, mean D = mean infestation density, mean P = mean infestation prevalence, mean I = mean infestation intensity. 95% bootstrapped, bias-corrected, confidence intervals in brackets. [a] All infested animals were infested with the same amount of ticks, thus a 95% CI for mean infestation intensity could not be calculated. [b] All animals were infested, thus a 95% CI for mean prevalence of infestation could not be calculated.

Table S4. Standardized model estimates with 95% confidence intervals for the analysis of infestation prevalence with non-feeding males. Models presented are the best performing hierarchical GLMMs with a binomial distribution. The model selected for the analyses is bold.

	Model 1			Model 2			Model 3			**Model 4**		
	Est.	95% CI		Est.	95% CI		Est.	95% CI		**Est.**	**95% CI**	
		Low.	Upp.		Low.	Upp.		Low.	Upp.		**Low.**	**Upp.**
Moose[a]	23.58	-47 495.02	47 542.19	26.53	-20 8162.8	20 8215.8	24.47	-75 763.85	75 812.80	**30.86**	**-20 124.57**	**20 125.18**
Red deer[a]	3.49	2.00	4.97	3.47	1.99	4.96	3.49	2.00	4.98	**3.30**	**1.96**	**4.64**
Roe deer[a]	1.35	0.15	2.55	1.37	0.16	2.59	1.35	0.15	2.55	**1.23**	**0.07**	**2.39**
Wild boar[a]	-3.70	-5.19	-2.21	-3.72	-5.21	-2.22	-3.69	-5.18	-2.19	**-3.73**	**-5.22**	**-2.24**
October 2019[b]	-0.17	-1.17	0.84	-0.15	-1.17	0.86	-0.18	-1.19	0.83	**-0.24**	**-1.11**	**0.64**
November 2019[b]	-2.90	-4.35	-1.45	-2.89	-4.34	-1.44	-2.92	-4.38	-1.46	**-2.65**	**-3.93**	**-1.36**
Freshness	-0.08	-0.53	0.37	-0.08	0.53	0.37	-0.08	-0.53	0.37	**—**	**—**	**—**
Male[c]	—	—	—	—	—	—	-0.10	-0.68	0.87	**—**	**—**	**—**
Young[d]	—	—	—	0.11	-0.66	0.87	—	—	—	**0.04**	**-0.72**	**0.79**
ΔAIC	0.00			2.10			2.12			**3.80**		

[a] Standardized correlation coefficients as compared to zero for fallow deer.

[b] Standardized correlation coefficients as compared to zero for October 2018.

[c] Standardized correlation coefficient for males as compared to zero for females.

[d] Standardized correlation coefficient for young as compared to zero for adults.

— Parameter was not included in the model.

Table S5. Standardized model estimates with 95% confidence intervals for the analysis of infestation prevalence (A) and intensity (B) with feeding females. Models presented are the best performing hierarchical GLMMs with a binomial distribution for infestation prevalence and with a truncated negative binomial distribution for infestation intensity. The models selected for the analyses are bold.

A	Model 1			Model 2			Model 3			**Model 4**			Model 5			Model 6		
	Est.	95% CI		Est.	95% CI		Est.	95% CI		**Est.**	**95% CI**		Est.	95% CI		Est.	95% CI	
		Low.	Upp.		Low.	Upp.		Low.	Upp.		**Low.**	**Upp.**		Low.	Upp.		Low.	Upp.
Moose[a]	1.04	-1.38	3.47	0.83	-1.65	3.30	1.04	-1.38	3.47	**1.11**	**-1.27**	**3.49**	0.82	-1.65	3.30	0.90	-1.50	3.30
Red deer[a]	0.66	-0.67	1.99	0.60	-0.78	1.98	0.65	-0.67	1.98	**0.78**	**-0.50**	**2.06**	0.60	-0.78	1.98	0.74	-0.59	2.08
Roe deer[a]	0.34	-1.20	1.88	0.27	-1.30	1.84	0.38	-1.23	1.98	**0.30**	**-1.24**	**1.85**	0.25	-1.39	1.89	0.24	-1.33	1.81
Wild boar[a]	-6.22	-8.05	-4.39	-6.32	-8.20	-4.43	-6.25	-8.11	-4.38	**-6.25**	**-8.03**	**-4.46**	-6.31	-8.20	-4.42	-6.33	-8.17	-4.48
October 2019[b]	0.22	-1.36	1.81	0.21	-1.40	1.82	0.23	-1.36	1.82	**-0.03**	**-1.50**	**1.44**	0.21	-1.40	1.81	-0.03	-1.54	1.47
November 2019 [b]	-2.65	-4.16	-1.14	-2.70	-4.26	-1.14	-2.64	-4.16	-1.13	**-2.67**	**-4.19**	**-1.16**	-2.70	-4.26	-1.15	-2.75	-4.33	-1.17
Freshness	-0.30	-1.00	0.39	-0.31	-1.04	0.41	-0.31	-1.00	0.39	—	—	—	-0.31	-1.04	0.41	—	—	—
Male[c]	—	—	—	0.44	-0.55	1.44	—	—	—	—	—	—	0.45	-0.58	1.48	0.57	-0.41	1.54
Young[d]	—	—	—	—	—	—	0.08	-0.89	1.05	—	—	—	-0.04	-1.06	0.97	—	—	—
ΔAIC	0.00			1.36			2.12			**3.19**			3.52			3.97		

B	**Model 1**			Model 2		
	Est.	**95% CI**		Est.	95% CI	
		Low.	**Upp.**		Low.	Upp.
Moose[a]	**-1.00**	**-2.02**	**0.02**	-0.99	-1.99	$-0.28 \cdot 10^{-3}$
Red deer[a]	**0.35**	**-0.07**	**0.77**	0.34	-0.08	0.75
Roe deer[a]	**0.01**	**-0.40**	**0.42**	0.01	-0.40	0.41
Freshness	**-0.05**	**-0.27**	**0.17**	-0.06	-0.28	0.16
Male[c]	—	—	—	0.07	-0.20	0.34
Young[d]	**-0.42**	**-0.68**	**-0.16**	-0.44	-0.71	-0.17
ΔAIC	**0.00**			1.96		

[a] Standardized correlation coefficients as compared to zero for fallow deer.
[b] Standardized correlation coefficients as compared to zero for October 2018.
[c] Standardized correlation coefficient for males as compared to zero for females.
[d] Standardized correlation coefficient for young as compared to zero for adults.
— Parameter was not included in the model.

Table S6. Infection prevalence of tick-borne pathogens in feeding *Ixodes ricinus* ticks from five studied ungulate species.

				Anaplasma phagocytophilum[a]		*Borrelia burgdorferi* s.l.[b]		*Borrelia miyamotoi*		*Babesia* spp.[c]	
		n_t	n_h	n_P	IP (95% CI)	n_P	IP (95% CI)	n_P	IP (95% CI)	n_P	IP (95% CI)
Fallow deer	Feeding larvae	48	22	38	0.79 (0.63-0.88)	2	0.04 (0.00-0.10)	1	0.02 (0.00-0.06)	1	0.02 (0.00-0.06)
	Feeding nymphs	1067	89	916	0.86 (0.84-0.88)	50	0.05 (0.03-0.06)	20	0.02 (0.01-0.03)	30	0.03 (0.02-0.04)
	Feeding females	551	99	495	0.90 (0.87-0.92)	52	0.09 (0.07-0.12)	5	0.01 (0.00-0.02)	42	0.08 (0.05-0.10)
	Non-feeding males	114	49	95	0.83 (0,75-0.89)	21	0.18 (0.11-0.26)	0	0.00	3	0.03 (0.00-0.06)
Moose	Feeding larvae	1	1	0	0.00	0	0.00	0	0.00	0	0.00
	Feeding nymphs	5	4	4	0.80 (0.00-1.00)	0	0.00	0	0.00	0	0.00
	Feeding females	48	14	34	0.72 (0.55-0.81)	2	0.04 (0.00-0.11)	2	0.04 (0.00-0.11)	4	0.09 (0.02-0.15)
	Non-feeding males	133	15	84	0.63 (0.53-0.70)	12	0.09 (0.04-0.14)	0	0.00	8	0.06 (0.02-0.10)
Red deer	Feeding larvae	5	2	5	1.00	1	0.20 (0.00-0.40)	0	0.00	1	0.20 (0.00-0.40)
	Feeding nymphs	100	20	93	0.93 (0.85-0.96)	5	0.05 (0.01-0.09)	0	0.00	9	0.09 (0.04-0.14)
	Feeding females	445	46	434	0.98 (0.96-0.99)	26	0.06 (0.04-0.09)	1	0.00 (0.00-0.01)	83	0.19 (0.15-0.22)
	Non-feeding males	286	48	232	0.81 (0.76-0.85)	44	0.15 (0.11-0.19)	1	0.00 (0.00-0.01)	18	0.06 (0.04-0.09)
Roe deer	Feeding larvae	2	2	0	0.00	0	0.00	0	0.00	0	0.00
	Feeding nymphs	130	22	85	0.66 (0.55-0.73)	6	0.05 (0.02-0.09)	2	0.02 (0.00-0.04)	32	0.25 (0.18-0.32)
	Feeding females	155	26	140	0.90 (0.85-0.94)	10	0.06 (0.02-0.10)	2	0.01 (0.00-0.03)	28	0.18 (0.12-0.24)
	Non-feeding males	75	22	59	0.79 (0.67-0.87)	10	0.13 (0.05-0.21)	1	0.01 (0.00-0.04)	8	0.11 (0.04-0.19)
Wild boar	Feeding larvae	0	0	-	-	-	-	-	-	-	-
	Feeding nymphs	7	7	4	0.57 (0.00-0.71)	0	0.00	0	0.00	0	0.00
	Feeding females	12	7	11	0.92 (0.66-0.92)	1	0.08 (0.00-0.25)	0	0.00	1	0.08 (0.00-0.25)
	Non-feeding males	15	3	5	0.33 (0.07-0.53)	1	0.07 (0.00-0.20)	0	0.00	0	0.00

n_t = number of tested ticks, n_h = number of animals the tested ticks came from, n_P = number of ticks positive, IP = infection prevalence with 95% confidence interval in parentheses. The 95% confidence intervals are 95% bootstrapped, bias-corrected, confidence intervals.

[a] Ten positive non-feeding males from fallow deer, five from moose and nine from red deer were sequenced as ectoype 1. One from moose, two from red deer and five from roe deer were sequenced as ecotype 2.

[b] For sequencing results see Table S8

[c] For sequencing results see table S11

Table S7. Standardized model estimates with 95% confidence intervals for the analysis of infestation prevalence (A) and intensity (B) with feeding nymphs. Models presented are the best performing hierarchical GLMMs with a binomial distribution for infestation prevalence and with a truncated negative binomial distribution for infestation intensity. The models selected for the analyses are bold.

A	Model 1			**Model 2**		
	Est.	95% CI		**Est.**	**95% CI**	
		Low.	Upp.		**Low.**	**Upp.**
Moose[a]	-3.98	-5.62	-2.35	**-3.94**	**-5.55**	**-2.33**
Red deer[a]	-2.88	-3.89	-1.87	**-2.83**	**-3.83**	**-1.84**
Roe deer[a]	-0.36	-1.57	0.84	**-0.66**	**-1.80**	**0.49**
Wild boar[a]	-5.39	-6.66	-4.12	**-5.09**	**-6.28**	**-3.90**
October 2019[b]	-0.88	-1.88	0.12	**-0.92**	**-1.91**	**0.06**
November 2019 [b]	-2.23	-3.36	-1.10	**-2.22**	**-3.33**	**-1.11**
Freshness	-0.32	-0.77	0.14	**-0.30**	**-0.75**	**0.16**
Male[c]	-1.14	-1.94	-0.33	**-0.92**	**-1.68**	**-0.16**
Young[d]	0.89	0.07	1.70	—	—	—
ΔAIC	0.00			**2.59**		

[a] Standardized correlation coefficients as compared to zero for fallow deer.
[b] Standardized correlation coefficients as compared to zero for October 2018.
[c] Standardized correlation coefficient for males as compared to zero for females.
[d] Standardized correlation coefficient for young as compared to zero for adults.
— Parameter was not included in the model.

B	**Model 1**			Model 2			Model 3			Model 4		
	Est.	**95% CI**		Est.	95% CI		Est.	95% CI		Est.	95% CI	
		Low.	**Upp.**		Low.	Upp.		Low.	Upp.		Low.	Upp.
Red deer[a]	**-1.46**	**-2.83**	**-0.08**	-1.35	-2.65	-0.05	-1.43	-2.73	-0.14	-1.35	-2.65	-0.05
Roe deer[a]	**-0.81**	**-1.63**	**0.01**	-0.70	-1.50	0.10	-0.79	-1.59	0.01	-0.70	-1.50	0.10
Freshness	**0.01**	**-0.29**	**0.30**	0.01	-0.28	0.31	0.01	-0.29	0.31	0.01	-0.28	0.31
Male[c]	—	—	—	—	—	—	0.09	-0.35	0.53	-0.01	-0.45	0.44
Young[d]	—	—	—	0.26	-0.13	0.64	—	—	—	0.26	-0.15	0.66
ΔAIC	**0.00**			0.55			2.09			2.84		

Table S8. Sequencing results from *Borrelia burgdorferi* s.l. positive ticks collected from ungulates.

		Borrelia afzelli	*Borrelia burgdorferi* s.s.	*Borrelia garinii*	*Borrelia valaisiana*	Not sequenced
Fallow deer	Feeding larvae	2	-	-	-	-
	Feeding nymphs	7	1	9	2	31
	Feeding females	5	1	3	-	43
	Non-feeding males	5	-	2	-	14
Moose	Feeding females	-	-	-	-	2
	Non-feeding males	1	-	-	-	11
Red deer	Feeding larvae	-	-	-	-	1
	Feeding nymphs	2	-	-	-	3
	Feeding females	2	1	-	-	23
	Non-feeding males	9	-	4	-	31
Roe deer	Feeding nymphs	5	-	-	-	1
	Feeding females	3	-	-	-	7
	Non-feeding males	1	1	-	-	8
Wild boar	Feeding females	-	-	-	-	1
	Non-feeding males	-	-	-	-	1

- No positive tick samples/No tick samples sequenced

Table S9. Standardized model estimates with 95% confidence intervals for the analysis of the infection prevalence of *Anaplasma phagocytophilum* in feeding nymphs.
Models presented are the best performing hierarchical GLMMs with a binomial distribution. The model selected for the analyses is bold.

	Model 1			Model 2		
	Est.	**95% CI**		Est.	95% CI	
		Low.	**Upp.**		Low.	Upp.
Red deer[a]	**0.01**	**-1.34**	**1.36**	$0.17*10^{-2}$	-1.35	1.35
Roe deer[a]	**-0.70**	**-1.67**	**0.27**	-0.72	-1.68	0.25
Male[b]	**—**	**—**	**—**	0.15	-0.62	0.92
Young[c]	**1.10**	**0.41**	**1.78**	1.04	0.31	1.77
ΔAIC	**0.00**			1.87		

[a] Standardized correlation coefficients as compared to zero for fallow deer.
[b] Standardized correlation coefficient for males as compared to zero for females.
[c] Standardized correlation coefficient for young as compared to zero for adults.
— Parameter was not included in the model.

Table S10. Standardized model estimates with 95% confidence intervals for the analysis of the infection prevalence of *Borrelia burgdorferi* sensu lato in feeding nymphs.
Models presented are the best performing hierarchical GLMMs with a binomial distribution. The model selected for the analyses is bold.

	Model 1			Model 2			**Model 3**		
	Est.	95% CI		Est.	95% CI		**Est.**	**95% CI**	
		Low.	Upp.		Low.	Upp.		**Low.**	**Upp.**
Red deer[a]	-0.11	-1.05	0.84	$-0.49*10^{-2}$	-1.08	1.07	**0.14**	**-0.98**	**1.26**
Roe deer[a]	0.01	-0.78	0.97	0.18	-0.81	1.17	**$0.23*10^{-2}$**	**-1.00**	**1.01**
Male[b]	—	—	—	-0.18	-0.84	0.48	**—**	**—**	**—**
Young[c]	0.89	0.30	1.47	0.93	0.23	1.63	**—**	**—**	**—**
ΔAIC	0.00			0.09			**2.89**		

[a] Standardized correlation coefficients as compared to zero for fallow deer.
[b] Standardized correlation coefficient for males as compared to zero for females.
[c] Standardized correlation coefficient for young as compared to zero for adults.
— Parameter was not included in the model.

Table S11. Sequencing results from *Babesia* ssp. positive ticks collected from ungulates.

		Babesia microti	***Babesia capreoli***	***Babesia venatorum***	***Babesia divergens***	***Babesia odocoilei-EU***	**Not sequenced**
Fallow deer	Feeding larvae	1	-	-	-	-	-
	Feeding nymphs	15	1	5	-	-	9
	Feeding females	27	1	4	-	-	10
	Non-feeding males	2	-	1	-	-	-
Moose	Feeding females	1	-	-	-	-	3
	Non-feeding males	3	-	-	-	-	5
Red deer	Feeding larvae	-	-	-	-	-	1
	Feeding nymphs	-	3[a]	2[a]	1[a]	-	5
	Feeding females	15	1[b]	13	1	2[b]	52
	Non-feeding males	6	-	3	-	-	9
Roe deer	Feeding nymphs	1	4[c]	2[c]	-	-	26
	Feeding females	6	6	-	-	-	16
	Non-feeding males	4	-	1	-	-	3
Wild boar	Feeding females	-	-	1	-	-	-

[a] This includes one nymph that was positive for *B. capreoli*, *B. venatorum* and *B. divergens*
[b] This includes on female that was positive for *B. capreoli* and *B. odocoilei-EU*
[c] This includes one nymph that was positive for *B. capreoli* and *B. venatorum*

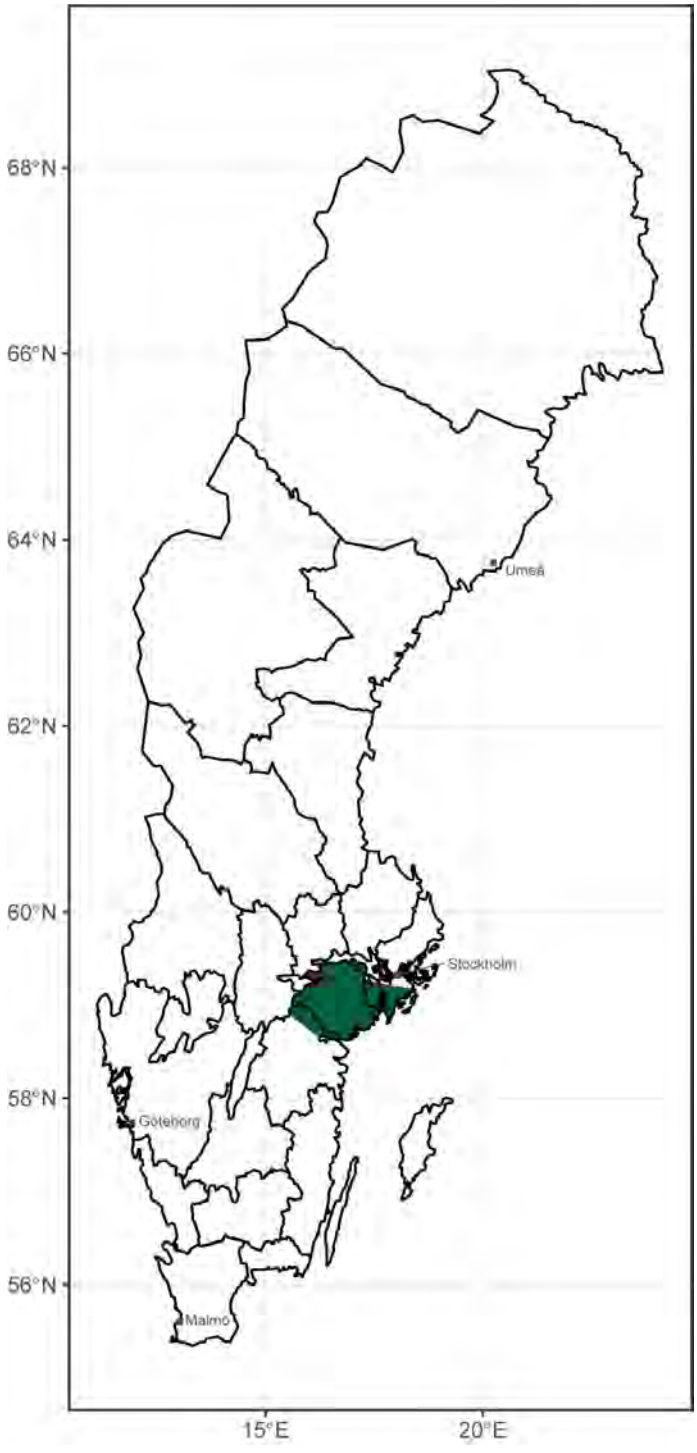


Figure S1. Map of Sweden with the study area in green.

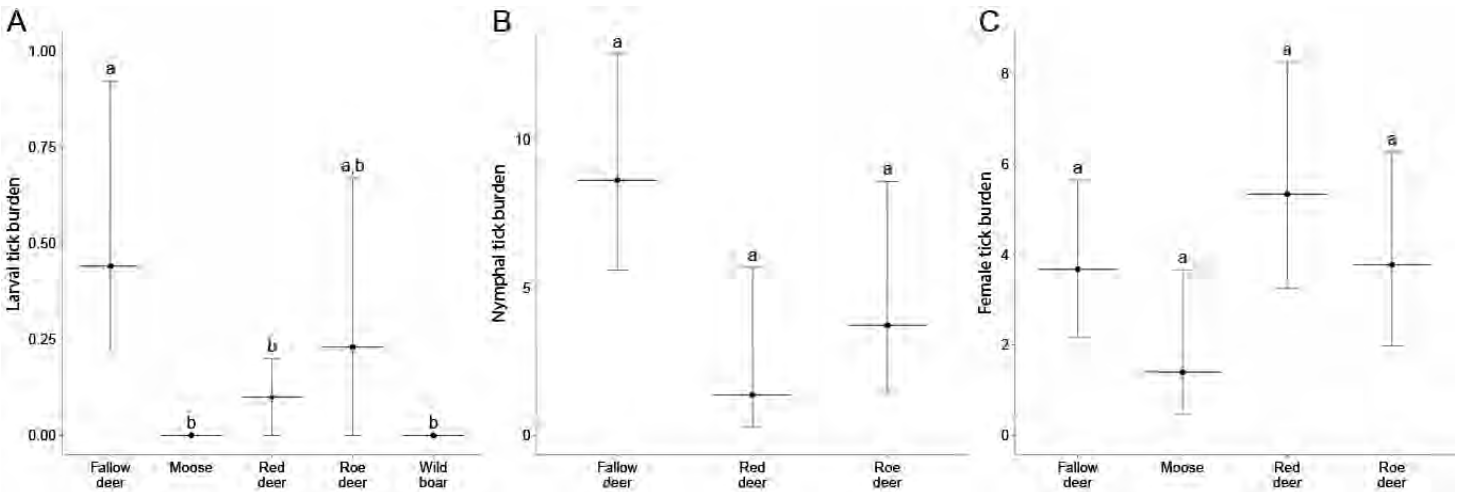


Figure S2. Larval (A), Nymphal (B) and Female (C) tick burden on the studied ungulate species. Tick burden, as calculated by formula 1, is given with 84% bootstrapped, bias-corrected, confidence intervals to show differences among ungulate species with a significance with an alpha value of 0.05.

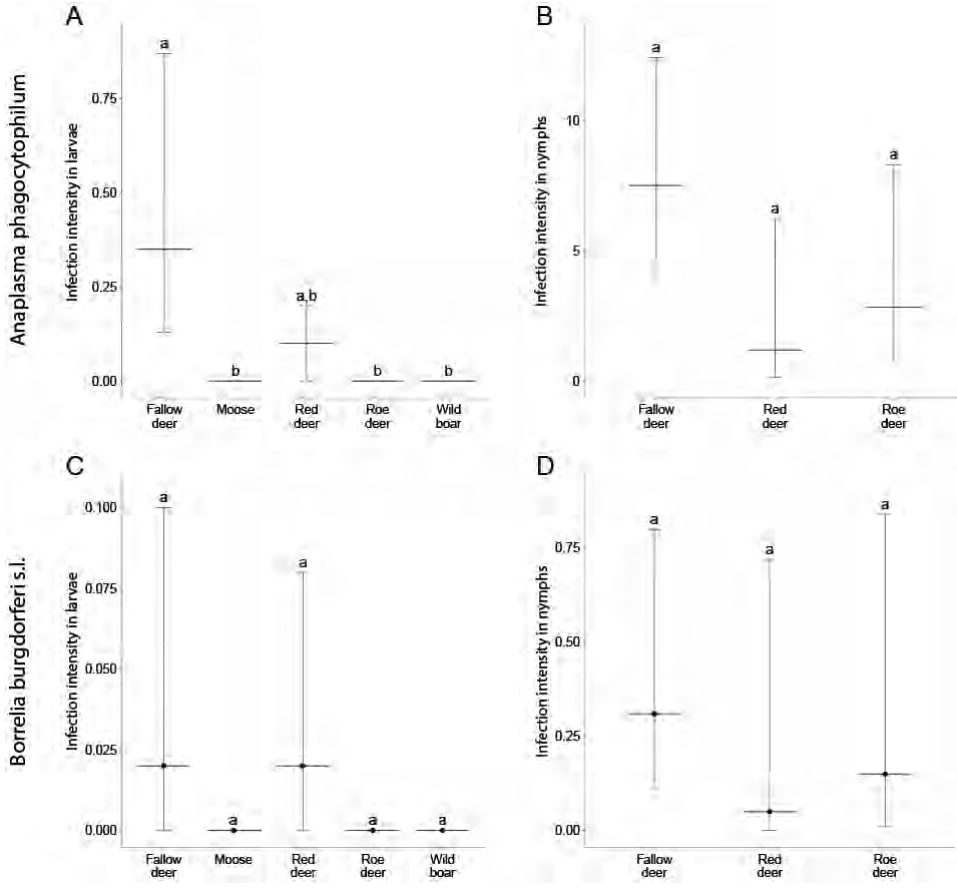


Figure S3. Infection intensity in larvae and nymphs from the studied ungulate species. Infection intensity, as calculated by formula 2, is given with 84% bootstrapped, bias-corrected, confidence intervals to show differences among ungulate species with a significance with an alpha value of 0.05. The four graphs show the *Anaplasma phagocytophilum* infection intensity in larvae (A) and nymphs (B) and the *Borrelia burgdorferi* s.l. infection intensity in larvae (C) and nymphs (D).

Wild ungulate species differ in their contribution to the transmission of Ixodes ricinus-borne pathogens.

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Additional file 2: Raw dataset

The additional file can be found on https://doi.org/10.1186/s13071-021-04860-w or scan the QR-code below to gain access.