The role of ungulates in *Ixodes ricinus* density in Europe

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Master Thesis, Utrecht University, Faculty of Veterinary Medicine

Supervisors:
Drs. N. Fabri
Prof. Dr. H. Heesterbeek

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Abstract

The tick, a bloodsucking arthropod, has a life cycle that requires a blood meal to moult to the next life stage, or to lay eggs. This blood meal is supplied by vertebrate hosts, and therefore a human can be a host as well. Because tick-borne pathogens can be transmitted when the tick attaches to a host, this poses a risk for humans and animals. That is why it is important to expand the knowledge on tick-host interaction and other factors that influence the tick life cycle, distribution and density. The aim of this thesis is to find out if and how ungulate densities are related to tick density, focussing on *Ixodes ricinus*. There are numerous studies that investigate one or more ungulate species and their densities with reference to tick-borne disease, and in some cases tick density. Unfortunately, the researchers do not reach a general consensus on a relation between tick density and ungulate density. The conclusion is that ungulates are of influence on tick density, and ungulate management techniques can be a tool to indirectly regulate tick density. This influence, however, requires more research that includes proper recording of ungulate density, tick density and climatic as well as environmental parameters.

Introduction

Vector-borne microorganisms can cause disease in humans and domestic animals. For several pathogens the tick serves as a vector, such as bacteria (e.g. *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Rickettsia spp.*), protozoa (e.g. Babesia) and viruses (e.g. Tick Borne Encephalitis virus, Crimean-Congo haemorrhagic fever virus)[1]. In fact, in the northern hemisphere, the larger part of vector-borne diseases are caused by tick-borne pathogens[2]. In the USA, 99% of all human vector-borne infections acquired in the country per year, are tick-borne[3]. According to Randolph, the European level of human infections is likely to be similar to that of the USA[2]. The European Environment Agency states that Lyme borreliosis (caused by the bacterium *Borrelia burgdorferi* sensu lato) is the most common vector-borne disease in the European Union (EU), with a known incidence of around 65.000 cases annualy[4]. Tick-borne encephalitis (TBE) and Lyme disease are the most important tick-borne diseases in Europe, both primarily transmitted by the tick *Ixodes ricinus* (*I. ricinus*).

Many people are aware of the risk of a tick bite while being in the woods. However, the risk is extended to walking in tall grass, parks and gardens. Higher mean temperatures, a changing environment and increased humidity facilitate tick survival and thus the spread of tick-borne diseases[5]. When use of land and urbanization changes, the frequency of contact between ticks (via wildlife), domestic animals and humans changes as well, increasing the risk of zoonotic diseases. It is clear that bringing tick exposure to a minimum is of veterinary and public health importance.

Ticks

Ticks are members of the *Arachnida* class; the spiderlike species. The subclass *Acari* includes two main families of ticks, the *Ixodidae* and the *Argasidae*, hard and soft ticks, respectively[6]. The life cycle of *I. ricinus* consists of three active life stages: larva, nymph and adult (Figure 1). All stages require blood from a vertebrate host for moulting to the following stage or, for female adults, to lay eggs. During each of these life stages, only one blood meal is taken. The lifespan of every stage counts eight to twelve months. Development is dependent on the number of light hours per day, temperature and host availability.

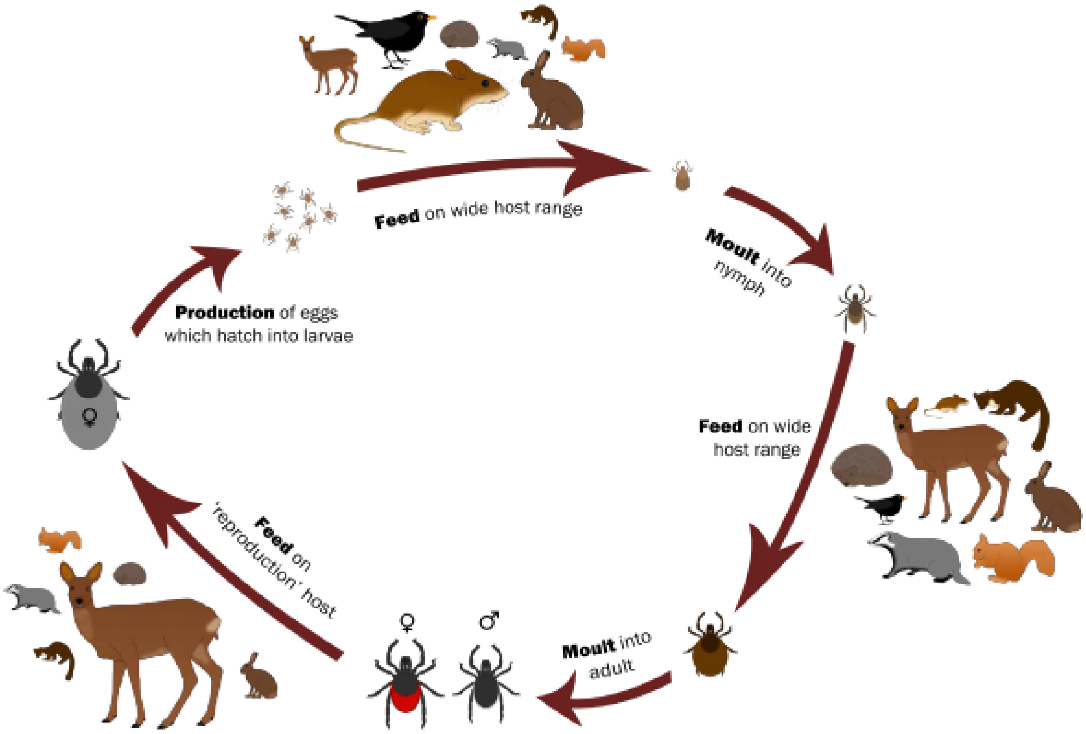

Figure 1: Schematic illustration of the Ixodes ricinus life cycle, made by Hofmeester[7]*. The I. ricinus life stages are larva, nymph and adult. I. ricinus immature stages feed on numerous host species, while adult female I. ricinus feed on a more limited range of hosts. Depiction of a species represents a group of species taxonomically related. Host size represents the estimated importance of the host taxon for that I. ricinus life stage.*

Ticks are sensitive to desiccation and therefore mostly found in forested places, seeking hosts primarily from February to November[8]. They find a host by the act of questing, where the tick climbs 20 to 70 cm up grasses or other structures, and waits with the front legs extended[9]. The immature stages of *I. ricinus* feed on a great array of host species, including rodents, hedgehogs, lagomorphs, mustelids and ungulates. Adult female *I. ricinus* feed on a smaller array of hosts, namely ungulates, hares and hedgehogs[6]. Ungulates are the most important hosts for adults[7]. The difference in host species the various life stages feed on, could be caused by differences in height of questing. Larvae and nymphs are more sensitive to drought, so moisture stress diminishes their questing behaviour and makes them stay closer to the ground[8]. This is an example of the impact of (micro)climate on stage-specific hosts.

Hosts

In the life cycle of the tick, hosts are vital. In between life stages, ticks spend time looking for a vertebrate blood meal. These vertebrates can be various types of animals, among which hoofed animals – the ungulates. Roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), elk (*Cervus alces*) and wild boar (*Sus scrofa*) all belong to this superorder. What makes the ungulate a potentially very relevant host species, is that they can host a large number of ticks and travel a much greater distance than, for example, rodents. On top of that, ungulates have expanded their geographical range in the past decades, with a worldwide abundance increase[10]. Because of a tick's parasitic nature, they are dependent on host availability. Characteristics of the hosts will affect parasite population dynamics, some ungulate species maybe more than others.

Tick distribution

A changing climate can make environments tick friendly. A survey study from Jaenson et al. shows that over the period of the early 1980s to 2008, *I. ricinus* has distributed northwards[11]. In the early 1990s, ticks were newly found in areas along the Baltic Sea northern coastline whereas in 2009, ticks were reported in many places in North Sweden for the first time. The authors explain the increased tick abundance firstly by the great availability of important tick hosts - roe deer in particular - and secondly by the warmer climate, permitting higher survival rates and distribution over larger geographical regions of both the tick and its host[11].

In the northern hemisphere, ever since the year 1950, the minimum temperatures have risen more than the maximum temperatures[12]. Temperatures in the winter have elevated more in comparison to the other seasons, in particular at higher latitudes. Spring commences a fortnight earlier than before the 1980s, and the duration of the vegetation season has enlarged[13]. These meteorological changes make for suitable tick climates and higher survival rates.

In 1999, Lindgren et al. examined whether the northward expansion of the *I. ricinus* habitat and the enlarged tick density were associated with the changes in climate. They found that the yearly number of days with minimum temperatures necessary for a vital tick life cycle were related to tick density[14]. The shifted distribution limit northwards and increased tick density are a combination of milder winters and prolonged spring and fall seasons – although they do not forget to mention a rapid increase in roe deer population.

The larger geographical distribution of ticks is not only latitudinal, but also altitudinal. Daniel et al. performed a study in 2009 on the vertical distribution of *I. ricinus* in the highest part of a Czech mountain area[15]. They found a large number of all developmental stages at 990-1300 meters above sea level (607 larvae, 614 nymphs, 8 females and 24 males). In a study from 1965 by Cerný et al., the upper limit of the ticks habitat was 700 meters above sea level[16]. Above this limit, the long-lasting unfavourable conditions extended the tick development in a manner that exhausted their energy supply[16]. The various stages died before they could moult or lay eggs, making it impossible to establish a population. So, Daniel et al. finding larvae, nymphs and adults demonstrates that in all the collection sites viable local tick populations are present, and the ticks were not coincidentally brought into higher altitudes via their hosts. Since the mountainous areas are often used for outdoor activities, the risk of vector-borne diseases increases.

New habitats

Significant transformations of land use can create new tick habitat, host habitat, and an increase in tick contact with the public. Take for example the fall of the Soviet Union. The dissolution caused a reform of agricultural practice, changing the landscape along the way[17]. Independence from the Soviet Union went hand in hand with an upsurge of TBE from 2- to 30-fold in central and eastern European countries. Low income and unemployment drove people to tick-infested forests to harvest wild foods. Deserted crop fields became viable for rodents and wildlife as hosts for ticks. A correlation was found between TBE-cases and poverty and household expenses on food[17].

Research aim

The aim of this research is to explore the role of ungulate densities in influencing *I. ricinus* density in Europe. This might give us more information about their role as transporting and feeding hosts for *I. ricinus* ticks. The aim of this thesis is to investigate what part ungulates play in the increasing distribution of ticks, and thereby the transmission of pathogens. The goal is to find out whether the various ungulate species differ in their role as host or distributor, or not. I hypothesize that ungulate densities are related to *I. ricinus* densities, and that this influence is different for various species. When this role is more defined in literature, it may be possible to predict what the effect is of different ungulate management options, such as culling or fencing, on the risk of humans acquiring tick-borne pathogens. This thesis will review background information and performed research on the different ungulate species. Then, the research techniques of the reviewed articles will be discussed. The expectation is that the outcome will support the effectivity of surveillance management of tick-borne zoonotic agents in wildlife.

Methods

For this research a literature study was conducted on the role of ungulates in tick density in Europe. The online database Pubmed was used, using different combinations of the terms "ungulates", "tick", "*Ixodes ricinus*", "deer", "roe deer", "Capreolus capreolus", "red deer", "Cervus elaphus", "wild boar", "Sus scrofa", "moose", "elk", "Alces alces", "fallow deer", "Dama dama", "tick borne", "tick borne disease", "vector", "vector borne disease", "distribution", "Europe". For each search, I combined "[ungulate, Latin name]" or "[ungulate, English name]" with "tick density". These terms yielded hundreds of articles, that were narrowed down to several dozens by only including articles that investigated ungulate density and/or tick density. For fallow deer, I only found one useful article. For wild boar and elk, the combination of "[ungulate] density" and "tick density" yielded no useful articles, so I looked for "[ungulate] density" and "TBE incidence". Further search databases that were used are Google Scholar and Scopus, using the same search terms. Additional publications were found through the references in other articles. Preferably only the articles with publication dates from the year 2000 and higher were used, except for basic information that has been established longer ago.

Review of ungulates on tick density

Hofmeester established that *I. ricinus* from all life stages were more abundant in areas with deer than without deer[7]. Research in distribution has been done by measuring different types of ungulates and their densities, and some found a relation[18] while others didn't[19]. This may be explained by a difference in effect of various ungulates. All ungulates experience energy-depleting yearly cycles of procreation, times of food deprivation and temperature stress[20]. In order to deal with these challenges, the ungulates possess a set of features and characteristics. These assets allow them to efficiently meet their food requirements, and at the same time keep competition with other animals to a minimum[20]. One example is a difference in the diet of large herbivores. There are three types: browsers (primarily woody and non-woody dicotyledons), grazers (primarily graminoids) and mixed feeders (browse and grass)[21]. Roe deer and elk are browsers, and red and fallow deer are mixed feeders, showing a great diversity in diet throughout the year. When the vegetation period ends, mixed feeder diets tend to resemble browser diets, performing a seasonal switch and adapting to the feeding type fitting local conditions[22]. Although there is intraspecific variation among ungulates[23], this may cause a difference in what type of vegetation they roam, encountering more or fewer ticks.

In the following section I will discuss five species of ungulates, namely roe deer, red deer, wild boar, fallow deer, and elk, with their particular ways of feeding, breeding, and difference in size. The presence and density of natural predators influences in what way ungulate numbers are controlled. While taking a further look in the influence of ungulates on ticks, I will try to pinpoint a possible primary species feeding the majority of *I. ricinus*, which will aid in developing species-specific management.

Roe deer

Roe deer (*Capreolus capreolus*) is the most plentiful ungulate in Europe, with a nearly constant dispersal over the continent and the UK[24]. Roe deer usually live alone or in small groups, herd sizes vary with season and habitat[25]. Population density is generally 15 to 25 animals per square kilometre, although up to 70 deer have been recorded in good quality environments[26]. They live in a large variety of habitat, but mostly in woods with clearings or woodland-field mixtures since these contain nourishment as well as shelter in close proximity. Territoriality can be observed all year round, but it is particularly pronounced during the rut[27]. Roe deer are known to travel, migrating more than 100 kilometres, so ticks can rapidly be dispersed over great distances[28]. Roe deer prefer dense vegetation and follow a diurnal rhythm of eating and resting, of which questing ticks may profit[24].

Vázquez et al. examined the skin of 367 roe deer shot during the hunting seasons (April-October) of 2007 and 2008 in Northwestern Spain[29]. The deer skin was taken off right after shooting, and the animals were separated according to their age (aged, adult, juvenile) and sex. The general prevalence of tick infestation was 83.1%. All of the 12,931 ticks found, belonged to the *I. ricinus* species, present in all infested animals, except for one *Dermacentor marginatus*. There were 9,102 adults, with a male/female ratio of 2.94, 2,777 were nymphs and 1,052 larvae. Preferred attachment places were the groin, axilla, neck and legs. The researchers found a mean intensity of tick infestation of 43.2 ± 49.85 per roe deer, ranging from 1 to 269. *I. ricinus* was found during the whole study period, with a 100% presence in spring and a 57.4% presence in autumn (Figure 2).

The immature life stages were more prevalent in spring and the first summer months, the adult prevalence was more spread over the year (Figure 3). Adult and nymph numbers show a peak in May-June, larvae numbers show minor peaks in June and in September. The researchers hypothesize that temperature may allow quick development of spring fed ticks, showing an autumnal peak after hot summers. A CHAID algorithm showed the host's sex as the primary influencing factor of nymph and adult tick prevalence in roe deer. Tick infestation was higher in males, older deer and in regions with higher roe deer densities, yet there was no significance in the differences. Unfortunately, Vázquez et al. did not measure tick density in the environment, so it is hard to draw conclusions on the influence of deer on tick density[29]. It does give insight in predictive parameters for tick burden.

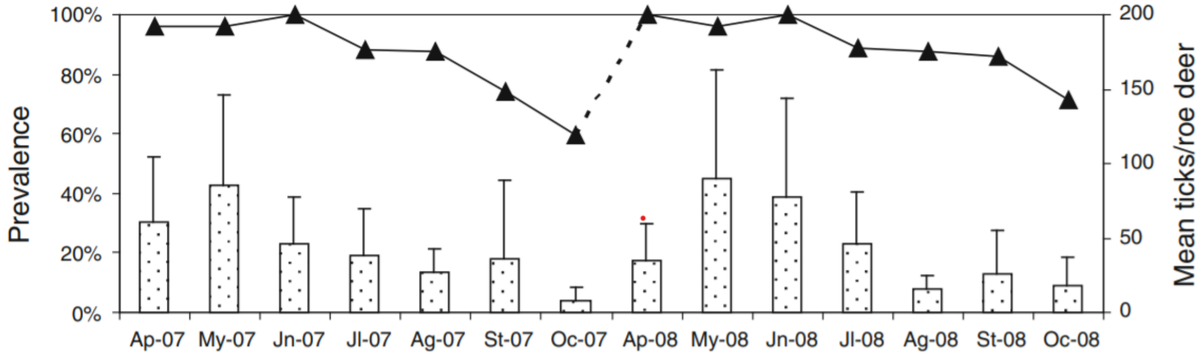

Figure 2: Prevalence per month and mean Ixodes ricinus infestation in roe deer found in North-western Spain. Figure from Vázquez et al.[29].

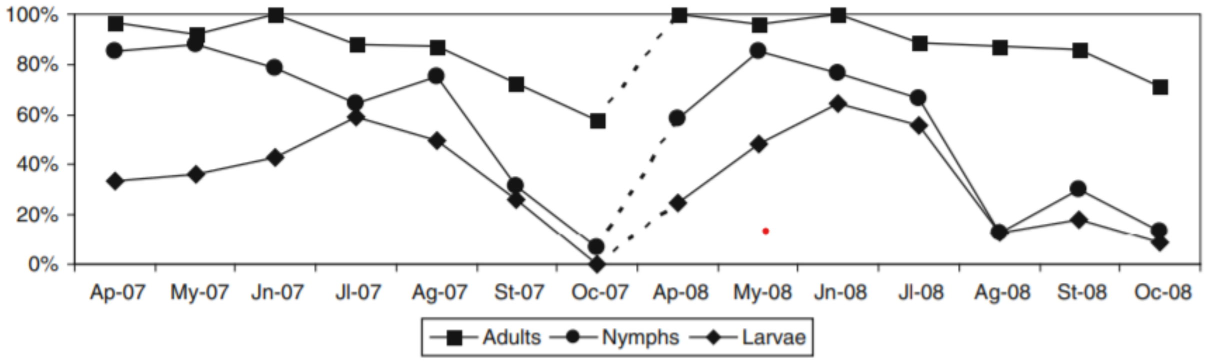

Figure 3: Prevalence per month of Ixodes ricinus life stages in roe deer found in Northwestern Spain. Figure from Vázquez et al.[29].

Another research that aims to capture predictive parameters, is from Vor et al.[30]. They assessed tick burden on roe deer with respect to age, physical condition, sex, deer density and season. In September 2007, May, July and September 2008 and in May and July 2009 they sampled ten days each period ticks of 142 culled roe deer in high TBE risk forests in Germany. The deer's heads and necks– the most infected parts according to their preliminary study – were investigated for a maximum of 30 minutes each. Roe deer density was measured using line transect methodology. A fixed circuit was driven two nights in a row, counting deer from the moving vehicle. Because of this particular method, the scholars consider their findings not as absolute density, but as an indication ranging from 2.4-9.1 deer/100 ha.
Per deer, tick quantity varied from 0 to 270 ticks, with a mean tick number of 65. There were significantly more adult ticks on older, heavier deer with larger hind feet. More ticks were found on male roe deer, but this outcome was biased by the male hunting season being May to October and female hunting season being in May and from September to January. There were no significant differences in September when males as well as females were hunted, with a tick count of 38 and 31 per deer, respectively. The parameters sex and roe deer hind foot length showed no significant effect on tick burden, age showed a negative correlation with *I. ricinus* larvae. The authors hypothesize that this preference for younger deer is because of more resting time and thinner skin. *I. ricinus* nymph, male and female counts peaked in May, most larvae were found in July. Significantly more nymphs were counted on deer from forest regions with higher deer densities. There is thus a correlation between nymph count and roe deer abundance.

Another research that found a positive correlation of *Ixodus spp.* nymph density and deer abundance is from Jensen et al[31]. In both spruce and non-spruce forest, tick density depended on soil water capacity as well as roe deer abundance. Sampling time, adult-nymph ratio and habitat type did not influence nymph density. The wetness of the cloth used for flagging ticks however, did have a significant effect on nymph density. A wet cloth reduced nymph numbers to 28% (±9%) in comparison with nymph capture using a dry cloth.

Carpi et al. collected ticks from 132 culled roe deer shot in the first two weeks of September 2004[32]. Right after shooting, the distal front legs were taken off, the only parts of the deer subjected to tick examination. The results suggest an association of geographic location with human TBE incidence and tick count on deer.
Rizzoli et al.[33] clarified principal driving factors for TBE cases in western Europe. Looking at datasets from four decades, they found no climatic variables related to an upsurge in TBE cases. They did find that a particular ratio of coppice to high stand forest, and roe deer density predicted numbers of TBE occurrence. High stand forest contributed more as a predictor than mixed forest, and roe deer density more than red deer density. Generalized linear models showed a significant negative relation between high stand forest and TBE cases, and a positive yet not significant relation with roe deer density and TBE cases.

Red deer

Red deer females and young live in small herds, and gather into bigger groups during the winter. Stags live solitary, except for in the summer when all-male herds can occur, and during the rut in late summer when red deer gather harems. Densities of 1 to 5 up to 15 individuals per square kilometre occur, with a maximum of 45 depending on habitat and supplementary feeding[25]. Red deer density correlates primarily with forest cover in many countries such as in Croatia and Poland[24]. Even though they are considered a woodland species, red deer have expanded their habitat recently, including mountain meadows and more open habitats.

Results from Tagliapietra et al. show a complex relationship between deer density and tick abundance[34]. Red deer density had a significant negative effect on questing nymph abundance, though only from the threshold value of 1.71 deer/km². Roe deer presence on the other hand, showed a positive effect on nymph abundance.

Qviller et al. reported higher *I. ricinus* questing density in the core than in the outskirts of red deer home ranges[35]. Tick density increased with the time red deer spent within a certain area, suggesting red deer move ticks across the landscape. The authors hold the view that the variation in red deer landscape use correlates with local tick distribution (at least on the Norwegian west coast). In earlier research, Qviller et al. hypothesize the reason for red deer migration is to create a spatial separation from ticks, avoiding tick abundance[36]. Resident red deer might circumvent tick hot spots locally – a behavioural defence against the ectoparasite. In addition to this hypothesis, Mysterud et al. provide evidence that tick loads are lower on red deer covering greater distances between the winter and summer areas[37]. Only the resident proportion of the red deer population seems to host ticks all-year-round, while the other proportion is unavailable for most of the questing season because of migration to higher altitudes.

Iberian red deer (*Cervus elaphus hispanicus*) and wild boar represent the paramount Spanish big game species. In Spain, the wild boar is present across the whole country, evenly spread with varying densities, while red deer is unevenly allocated[38]. The big game species are of economic interest regarding hunting, and are therefore managed more and more. Fencing and extra feeding led to higher boar and deer densities causing concern for vector-borne diseases. This is why Ruiz-Fons et al. sampled 475 red deer and 284 wild boar during 1999 to 2005[39]. They collected ixodid ticks from 431 red deer and 142 wild boar. 55% of the animals were derived during the hunting season from October to February. A total of 6336 ixodid ticks were collected, only 3,7% in red deer and 0,1% in wild boar belonged to the *Ixodes ricinus* species. *Hyalomma marginatum marginatum* was the most common species, making up 63,7% and 68,7% of the total tick harvest in red deer and wild boar, respectively. Interestingly enough, Ruiz-Fons et al. found that adult stages of various tick species parasitized red deer, but no wild boar residing in the same area, and the other way around. They hypothesize that either there is competitive behaviour between species, or that life cycles have adapted to minimize host competition.

Wild boar

The wild boar, or Eurasian wild pig, lives in groups of around twenty animals. Male adults tend to live solitary outside the procreation season. Being omnivores, wild boar consume nearly anything from fruits, grass and nuts to small reptiles and invertebrates[25]. Per 100 square kilometres 0.1-17.9 individuals can be found, varying immensely throughout Europe. Wild boar have the tendency to come closer to villages when food is scarce, and can end up as roadkill in the process. In Germany they make up 9% of all traffic accidents with ungulates[27].

In the Czech Republic, they seem to be related to TBE incidency[40]. Since human TBE cases and game populations showed an upward trend, there was interest in examining a possible relation between the two. The study period ranged from 2003 to 2011, in which the researchers focused on two game species most common in the Czech Republic: roe deer and wild boar. Within the study period, 6213 TBE cases were reported, and 1.062.308 roe deer and 989.222 wild boar were culled. A significant positive association between forest size and agricultural area, and TBE numbers was found, as well as an association in TBE numbers and culled wild boar. This relation was not found for culled roe deer, perhaps – the authors think – because wild boar are more dispersed across the forest and agricultural areas. Wild boar also come in contact with small rodents while digging up ground.

As stated by Ruiz-Fons et al. in the section on red deer, ticks may have a host preference when boar and other tick hosts share common habitats[39]. Although it looks like there is an association between TBE number and culled wild boar, this is not the same as an association between tick density and wild boar density.

Fallow deer

Sizewise fallow deer (*Dama dama)* stands in between roe deer and red deer[41]. The name fallow comes from the pale brown fur of the deer[24]. A remarkable trait of the animal, is that bucks older than three years develop broad, shovel-shaped antlers. The mixed feeders live in large single-sex groups of up to 70 individuals. The males often go solitary during the rut[42].

Fallow deer is a species that, regarding tick density, has not been fully studied. Very recent Scottish research by Dickinson et al. aimed to quantify the relationship between deer density and questing *I. ricinus* nymph density[43]. At each of the 17 sites studied (total of 120 km²), line transects were placed in North-South orientation at 200-meter intervals. There were twenty observation points at equal intervals marked along the transect. The testing took place on three scales: observation level as the finest scale with estimates per sampling point, transect level as medium scale with estimates per transect, and site level as the broadest scale with estimates per site. Nymph density was measured by collecting ticks during the main questing period – May to July – in 2016. At the twenty observation points, 10 m² blanket drags with 1 m² blankets were conducted, once per site. Ground temperature and humidity were measured at the start and end of each drag. Height of vegetation, density and type were documented at three intervals along the 10-meter transect, and then averaged.
Deer density was measured by dung sampling of line transects in winter and summer of 2016. An observer walked each line transect marked at 50-meter intervals, recorded each deer dung and identified the species. Then, the observer marked the dung with biodegradable tape to prevent double counts. In summer, during the second count, height of vegetation, density and type was scored to determine the effect of vegetation growth on dung detection in the summer months.

Deer density could not be measured at observation level, so the number of deer dung along the transect was used. Deer density at transect and site level, and in winter- and summertime were combined to define average deer density. 95% of the dung came from fallow deer, only 5% came from roe deer or sika deer.
At transect level, deer density was a significant predictor of nymph's questing density. At site level, deer density and nymph density held a positive association. Nymph density increased with higher temperatures and lower humidity, and with lower vegetation height and density. The summer estimates positively predicted questing nymph density, whereas winter estimates did not. Deer density estimates were lower in summertime than in wintertime, at all studied levels.

Elk
The Eurasian elk (*Alces alces*) occurs in a range from Scandinavia and eastern Europe through Siberia to the Yenisei river[27]. It is the largest living deer, and eight subspecies are known to exist on the northern hemisphere[25]. This browse feeder is active throughout the day, with activity peaks at daybreak and dusk. Males and females only mix during the rut in September or October and are living largely solitary the rest of the year[25]. Elk densities tend to be low, ranging from 0.7-1.2 animals per km² depending on the used counting method (hunter observations or pellet group counts)[44].

Results from Tonteri et al. establish local TBEV circulation in elk in known endemic areas[45]. Nine out of 1213 hunter-harvested elk were seropositive for TBEV, and only one seropositive elk had been shot in a non-TBE endemic area. The researchers suggest elk could serve as sentinels for TBEV, and risk indicators for human TBE infections in areas where deer are not widely spread.

Although research that investigates the influences of multiple ungulates on ticks are rare, Jaenson et al. thought it worth their while[46]. Roe deer, red deer, fallow deer, wild boar and elk were all included in the research. Jaenson et al. used annual statistics on the numbers of the wildlife species harvested by hunters as indication of animal density. Abundance data of their predators, red fox and the lynx, were also included. On average, roe deer and red deer showed positive, significant covariance with human TBE incidence. Elk and fallow deer showed negative significant covariance, and wild boar, lynx and red fox showed no covariance at all. The positive value means that high numbers of culled animals in one year resulted in higher human TBE incidence the year after (Figure 5). They year time lag was chosen because *I. ricinus* usually become infected with TBEV as larvae and may transmit the virus as nymphs eight to twelve months later.
A combination of all game species analyses showed a significant relation between culled animals and TBE incidence. The analyses without a one-year time lag did not yield any significant results. The authors conclude that one or more aspects of roe deer and red deer abundance explain TBE incidence the year after.

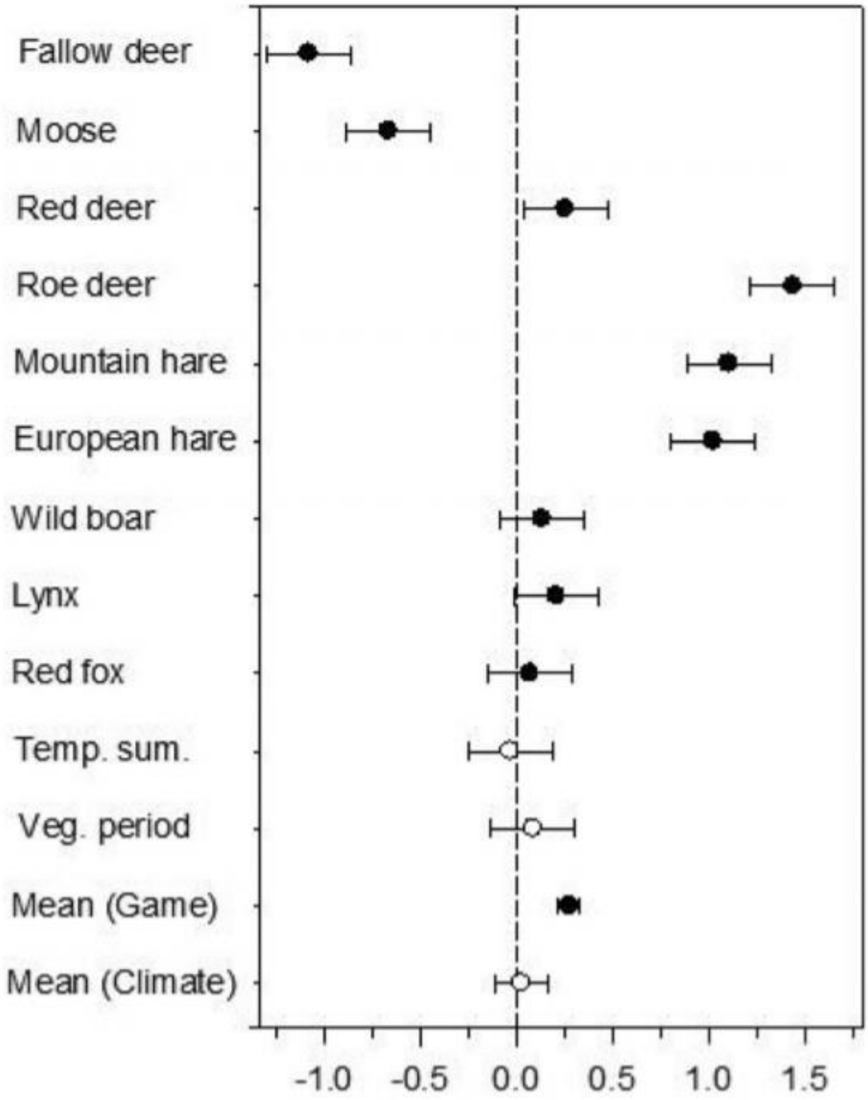

Figure 5: Meta-analysis on the effect of culled wild animals (filled symbols) and climatic variables (open symbols) on TBE incidence from Jaenson et al.[46]*. A positive value means a high number of culled animals or high values of the environmental parameters in one year, resulted in increased human TBE cases the year after. Dots stand for mean effect sizes, the bars for the 95% confidence intervals. If the bars reach or cross the zero line this means that there was no significant effect of culled animals or environmental parameters on TBE incidence.*

Summarizing, there seems to be a relation between roe deer density and tick density, especially nymph density, although this is dependent on multiple factors such as vegetation, wetness of the cloth and age of the deer. It appears red deer show behavioural adaptations to avoid tick bites, and tick densities are higher in areas where red deer reside. Culled wild boar and TBE number seem to hold an association, although this cannot be directly interpreted as an association between tick density and wild boar density. Fallow deer density and nymph density seem to hold a positive association according to Dickinson et al., but Jaenson et al. state the opposite[43,46]. The results of the influence of elk density on tick density are scarce, and a conclusion cannot be drawn.

Synthesis and discussion

Due to overexploitation at the end of the nineteenth century, deer numbers and ranges declined dramatically. Protection of deer followed, via restricted hunting seasons and game laws. Consequently, deer population increased rapidly over the past 75 to 150 years[47]. Within a century, deer management had shifted from preserving and augmenting populations, to grave concern about the growing impact of these animals on environmental constituents. The main view on *I. ricinus* density, is that it is determined by deer abundance, but studies on the matter are not consistent[18,31].

Although there is evidence showing more deer mean more ticks[30], fewer deer can lead to tick amplification and thereby more TBE cases - at least in small exclosures[48]. Van Buskirk and Ostfeld hold the view that deer density and questing nymphal *I. ricinus* density follow a threshold relationship[49]. This view is supported by Hofmeester[7], as no tick life stage increased in density with relative deer abundance. Solely in areas where roe deer were the only deer, tick density and relative deer abundance were related. The problem in comparing the evidence, is the variety in research techniques. Research that only measures ticks[14], or only measures deer density[29,33] cannot be easily juxtaposed.

Measuring ungulate density

Not all studies have measured ungulate density in the same way, let us take a look at the different applied techniques. Some researchers, such as Jaenson et al., consider the hunting data to be the most useful and reliable to determine the relative ungulate densities. They assume that the numbers shot are directly proportional to the true densities [46]. Although they show articles supporting their theory, using hunting numbers could also create more bias. The temporal variability of shot wildlife is influenced by hunting activity variating in season and year and is therefore selective and not random. The study from Ruiz-Fons et al. is a good example of hunting season bias: the majority of the animals were sampled for ticks during the hunting season[39].

Another technique for measuring ungulate density is counting dung[43]. This sounds more accurate than using numbers of culled animals, but one would preferably combine this data with camera trapping data[7] so the passage rate is determined. A different approach that aims to measure ungulate density, is counting while driving a jeep through the woods, like Vor et al. have done[30]. One could argue how useful this is, considering that the animals are wild and tend to stay away from humans.

The necessity of measuring ungulate densities throughout the year becomes clear when animals are seasonally migrating. Because of the migration, the amount of animals available differs throughout the year[37]. Only the resident part of the population will be available as year-round hosts to ticks. A large part of the population is unavailable to ticks for the majority of the tick questing season, due to seasonal migration to higher altitudes. Winter estimates of deer density are not useful in predicting questing nymph abundance, while summer estimates are[43]. This illustrates the importance of rightfully choosing ungulate density measurements as well as tick density measurements, as they are influencing each other.

Measuring tick density

There is research, such as from Dickinson et al., that aims to count every ungulate on the perimeter[43], and research that only examines parts of (culled) animals[32,30]. As there are studies that only look at a relation between tick-borne diseases and ungulate density[33], and not between tick density and ungulate density, the question arises if this is an adequate strategy. As described earlier, Tonteri et al. found TBEV-positive elk in endemic areas, but did not establish the tick or elk density in the region[45]. TBE-incidence and TBEV-seropositive elk are not the same as elk density. The question is whether TBE-incidence can be used as a determinant, when the number of infected ticks is unknown. The reason I added the articles on TBEV in relation to ungulate density and ungulate infection grade, is because this also shows ungulates are not just 'dilution hosts', but actual part of the problem in transmitting disease[48]. Some, like Rizzoli et al., even state that TBE-incidence in humans is statistically associated with roe deer density[33]. The ungulates can be TBEV serving hatches when ticks are co-feeding, proven by seropositivity in the animals[45].

While Jaenson et al. found a positive correlation between roe deer and red deer abundance and TBE-incidence, they argue that the nymphs usually transfer TBE - but it takes two to three years (in southern Sweden) for an egg to develop into a nymph[46]. The one year time lag they implemented may be too short. They justify this by saying the majority of roe deer shot are bucks over one year of age. These bucks are likely to have provided the tick population with blood for more than one year, agreeing with the tick's life cycle. Another reason may be that the development of egg to adult occurred faster because of suitable microclimate. Jaenson et al. would not know this, since they did not drag ticks or measure temperature and humidity during their study.

As Moestrup Jensen et al. concluded, the wetness of the cloth used for tick dragging or flagging, is of influence on the number of caught ticks[31]. Some researchers measure the temperature and relative humidity on site every time they go tick dragging[43] and some researchers take temperature data from meteorological institutes nearby[46]. Because microclimate may be of influence on the number of ticks grabbing on to the cloth or flag, institutional data seems not to be precise enough.

Although Kriz et al. performed research on almost a million wild boar, tick density was not taken into account[40]. It is possible that in the Czech Republic a lot of ticks happen to be infected with TBEV, meaning TBE-numbers could be (practically) equal to tick density.

Conclusion

The main objective of this review was to better understand the role of different ungulate species on *I. ricinus* populations. As Randolph accurately states: the long, slow life cycle of the tick poses a challenge to tick ecologists, and any scientist willing to elucidate the tick-host relationship[9]. Ungulates have a great influence on other organisms and natural processes, although this review shows a variety in research outcome. With the present level of research, it is not possible to appoint a primary ungulate with the most influence on tick density. Scientists should profoundly investigate efforts to monitor, understand, and reduce the impact of ungulates on ecosystems[10]. When ungulate presence is more important than ungulate abundance, culling can prove less useful. Fencing would be a better option, especially for reducing tick densities in areas with high recreational pressure. On the other hand, when considering the animal's perspective, red deer and other migrating animals might have higher tick infection grades when fences prevent them in their spatial distribution. Ungulate management should be based on the residing ungulate species and season.

As regards to further research, Vor et al. suggest including more specific habitat characteristics such as vegetation height, canopy cover and soil moisture to better predict tick densities[30]. Schwarz et al. state that nature reserves can be equipped with analyses of environmental factors to predict *I. ricinus* densities, to inform tourist about high tick density hotspots[50]. There is research that looks at multiple ungulate species at the same time[39], but not one that takes all ungulates into account, measures tick density in an adequate way and considers the climatic factors and the ecosystem.

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