

## 6. The role of large herbivores in *Ixodes ricinus* and *Borrelia burgdorferi* s.l. dynamics

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### Abstract

Large herbivores are the most important reproduction hosts for *Ixodes ricinus*, and, as such, play a major role in maintaining tick populations. As one individual deer can already feed many females during the tick season, we propose that the relationship between deer density and tick density can best be described by a step function rather than a linear function. At high densities, herbivores may negatively affect tick numbers through their effects on vegetation structure and composition by creating and maintaining a short and open herb layer, reducing the shrub layer and decreasing the thickness of the litter layer. These effects may also have a negative effect on rodent densities. Domestic herbivores as added grazers will likely not have a major added effect on tick numbers but at high density they may have, both through their effects on the vegetation and because they may negatively affect the habitat use of the wild ungulates through competitive interactions. Large herbivores are mainly incompetent, in the sense of not-transmitting the parasite *Borrelia burgdorferi* s.l. to ticks, but to what extent this will affect the density of infected nymphs in a system is dependent of the host community as a whole and cannot be predicted from the density of the large herbivores alone.

**Keywords:** *Borrelia burgdorferi* s.l., competence, deer, density, domestic herbivores, grazing effects, *Ixodes ricinus*, prevalence

### Introduction

*Ixodes ricinus* in Europe is the main vector for the bacterium *Borrelia burgdorferi* s.l. which can cause Lyme borreliosis in humans. The number of ticks present or active at any time, and the infestation with parasites, is influenced by many factors. As ticks spend more than 90% of their time in the top soil and in the lower parts of the vegetation, environmental and habitat characteristics like temperature, humidity and presence and thickness of the litter layer are key determinants of survival rate and activity levels. The presence of suitable hosts for the different tick stages is equally important. In this chapter we will elucidate the role of large herbivores in the *I. ricinus* tick cycle and, to a lesser extent, we will pay attention to the role of large herbivores in the *B. burgdorferi* s.l. life cycle. The large herbivores we will discuss are mainly the more common and abundant deer species roe deer *Capreolus capreolus* and red deer *Cervus elaphus* because they are thought to be the most important hosts for the adult tick stage in western Europe (Hofmeester *et al.* 2016). Apart from these wild ungulates, we will also discuss the role of some species of domestic herbivores, mainly sheep, as many areas are being grazed by them, either for conservation purposes or in a rangeland setting (Van Wieren and Bakker 2008). In many cases domestic stock is present sympatrically with wild ungulates but in some cases they are the only large herbivores present and as such are important for maintaining tick populations. Insight in the role of large herbivores can also be used to try and find suitable and feasible intervention options to reduce tick abundance (see also chapter sheep mopping).

## Ticks on large herbivores

Counting ticks on large furry animals is a tedious job but nevertheless Hofmeester *et al.* (2016) were able to compile a large database with tick counts from various study areas throughout Europe. In Table 1 an overview is given from the counts on various large herbivores. In most cases prevalence of tick infestation was high and most animals carried ticks. The distribution of the various tick stages on the animals is in general different from the distribution in the habitat. Nymphs and adults prevail and in quite a number of cases more adults have been counted than nymphs. Together with the finding that small and medium sized hosts generally only feed juvenile ticks (Hofmeester *et al.* 2016), it stresses the fact that large herbivores are the main hosts for adult ticks and, as such, are a key element in sustaining tick populations. They are reproduction hosts. Nevertheless, larvae also feed on large herbivores, sometimes in large numbers, while it is likely that despite intensive searching, larvae are easily overlooked.

Not many counts have been done on domestic animals but they carry ticks as well.

The results of some studies suggest that sheep and cattle might carry fewer ticks than wild species like deer. In sympatric living wild and domestic species, wild animals were found to be more infested (and with a wider variety), than domestic animals (Dominguez-Peñafiel *et al.* 2011). But also high densities have been reported. Milne (1943) counted female ticks on 10 groups of hill sheep in England. Prevalence varied from 0.45-1.0, the mean number of females varied from 0.85-85.6 and some sheep were infested by more than 150 females.

In a study of L'Hostis *et al.* (1994), the number of females per cow ranged from 1-186. The low numbers in some grazer populations can probably partly be explained by the fact that much grazing takes place in more open landscapes, which are generally less tick-infested than woodland habitat (Boyard *et al.* 2008).

In Table 1 mean numbers are given but it is well known that tick numbers on animals vary widely and that the distribution is heavily skewed. Milne (1943) was the first, together with RA Fisher, to discover that, although at lower tick densities the data could well be described by a Poisson distribution, at higher densities a negative binomial distribution was more appropriate. Tälleklint and Jaenson (1997) found heavily skewed distributions of larvae in roe deer and hares. Although we might expect somewhat less skewed distributions of nymphs and females, as larvae have a much more clumped distribution than nymphs or females, very skewed distributions have also been reported for females (L'Hostis *et al.* 1994, Milne 1943).

Variation in infestation levels can be thought of to arise from a variety of sources:

- The (patchy) distribution of ticks in the habitat.
- The distance travelled by the animal/area covered.
- The physical and physiological state of the animal.

We do not know of any study where all these factors have been taken into account to explain infestation rates. Yet some differences between different classes of animals have been found. Milne (1947) counted more ticks on ewes lower in body condition. He also found a clear effect of body size. The larger ewes had more ticks than rams, who had more ticks than lambs. In one of our studies ticks were counted on 17 roe deer (9 does, 8 bucks), randomly chosen from five different areas. Boxplots of the tick counts are given in Figure 1.

Table 1. Tick, *Ixodes ricinus*, counts on large herbivores from different areas in Europe (ND = not determined).

Area	Host species	n	Prevalence	Total n ticks	Mean number			Reference	
					All stages	Larvae	Nymphs		Adults
Norway	roe deer	289	0.60	ND	ND	ND	ND	Handeland et al. (2013)	
Thuringia, Germany	roe deer	103	0.67	4,027	39	ND	ND	Heyl and de Mendonca (2011)	
Southern Hungary	roe deer	35	ND	132	4	0	1	3	Hornok et al. (2012)
Gottingen, Germany	roe deer	80	1.00	5,159	64	11	24	30	Kiffner et al. (2010)
Brandenburg, Germany	roe deer	67	ND	833	12	9	4	ND	Matuschka et al. (1993)
North-Western Spain	roe deer	154	0.83	5,954	39	3	10	26	Pato et al. (2013)
Sweden	roe deer	37	ND	14,985	405	265	93	47	Tälleklint and Jaenson (1997)
Galicia, Spain	roe deer	367	0.83	12,930	35	3	8	25	Vazquez et al. (2011)
Germany	roe deer	142	0.86	7,576	53	2	27	24	Vor et al. (2010)
Byalistok province, Poland	roe deer	20	0.80	ND	2	ND	ND	ND	Wegner et al. (1997)
Norway	red deer	290	0.55	ND	ND	ND	ND	ND	Handeland et al. (2013)
Southern Hungary	red deer	32	ND	309	10	0	0	10	Hornok et al. (2012)
Brandenburg, Germany	red deer	147	ND	5,512	37	28	9	ND	Matuschka et al. (1993)
the Netherlands	red deer	38	ND	2,539	67	8	16	43	Pacilly et al. (2014)
Byalistok province, Poland	red deer	12	1.00	195	16	ND	ND	16	Wegner et al. (1997)
Brandenburg, Germany	fallow deer	115	ND	7,207	63	50	13	ND	Matuschka et al. (1993)
the Netherlands	mouflon	22	ND	284	13	3	5	5	Pacilly et al. (2014)
the Netherlands	wild boar	9	ND	137	15	8	6	1	Pacilly et al. (2014)
Byalistok province, Poland	wild boar	7	0.14	7	1	ND	ND	1	Wegner et al. (1997)
Norway	moose	440	0.27	ND	ND	ND	ND	ND	Handeland et al. (2013)
Sweden	moose	7	ND	4,101	586	290	173	122	Tälleklint and Jaenson (1994)
Southern Hungary	goat	110	ND	272	2	1	0	2	Hornok et al. (2012)
Southern Hungary	sheep	375	ND	15	0	0	0	0	Hornok et al. (2012)
England	sheep	6	1	215	36	64	36	8	Ogden et al. (1997)
France	cattle	110	0.7	1,163	11	ND	ND	11	L'Hostis et al. (1994)

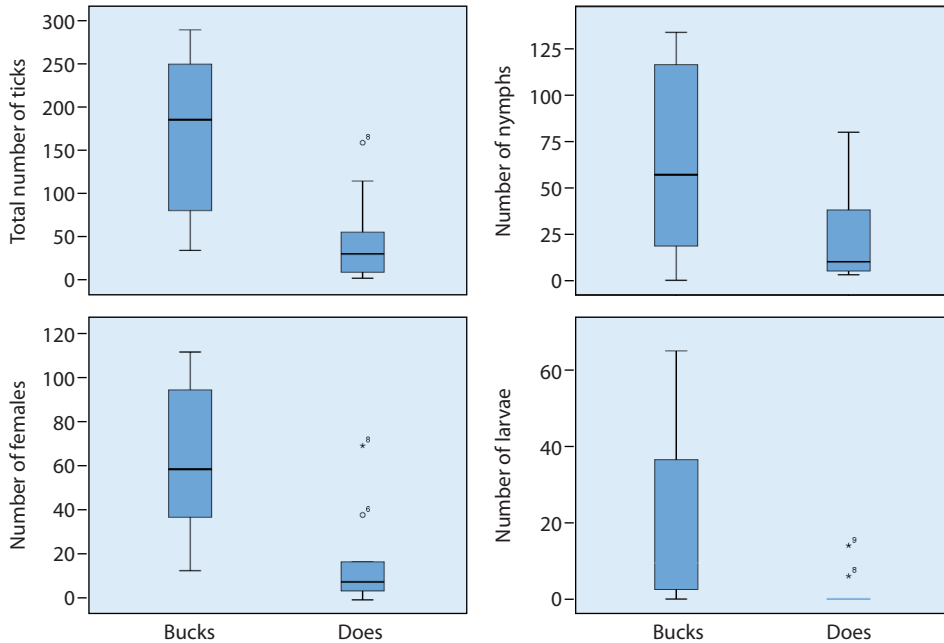


Figure 1. Tick counts on roe deer bucks (8) and does (8) from 5 different areas in the Netherlands (2012).

Bucks carried significantly more ticks of all stages than does. Male and female ticks were found attached to each other 163 times, 63% of the males were attached to a female and 25% of the females to a male. The sex difference cannot easily be explained. Roe deer bucks and does do not differ much in size. Perhaps the most likely explanation is that males tend to move much more than females and so have more chance of encountering a tick.

### The effect of herbivore density

If large herbivores are so important as reproduction hosts, we can expect tick densities to be very much reduced when large herbivores are absent. We compared tick densities of areas with and without large herbivores (Figure 2). Tick densities in the areas without large herbivores are very low indeed (T.R. Hofmeester unpublished data). Ticks are not completely absent as there will always be some medium-sized host species (e.g. birds, hares, martens) that feed a number of adults and so maintain a tick population at low density. We can also expect a positive relationship between herbivore density and tick density. A number of studies suggest a linear relationship between deer density and tick density (Jensen and Jespersen 2005, Rand *et al.* 2003, Sprong *et al.* 2012). The problem with most of these studies is that crude estimates of deer densities were used and that the analyses were covering very large areas (countries). Especially what happens at low deer densities is not clear.

We studied the relationship between *I. ricinus* densities as measured by blanket dragging, and the number of deer active as measured by camera trapping, in twenty forest plots of 1 hectare. At this smaller scale, it is clear that this relationship is non-linear (Figure 2).

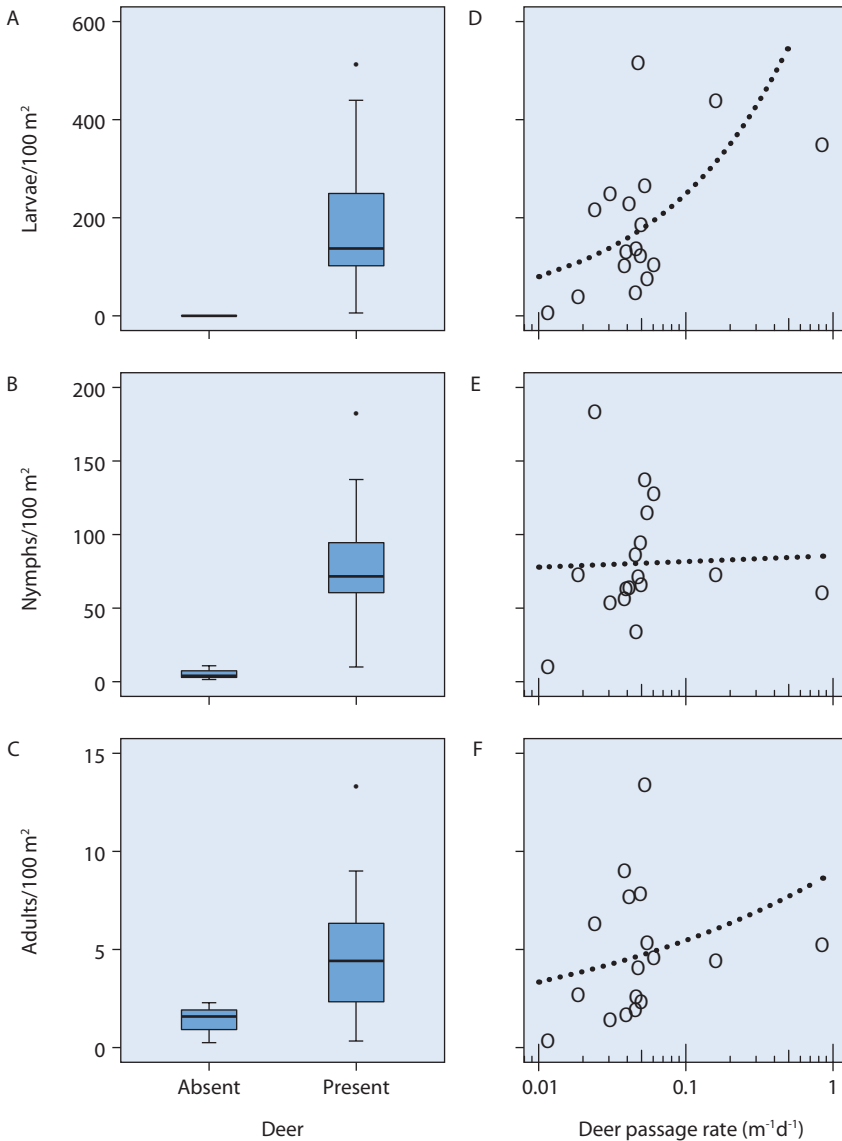


Figure 2. The density of the sheep tick *Ixodes ricinus* differed significantly ( $P < 0.01$ ) between 17 forest plots with deer and 3 plots without (A-C), but did not significantly increase with deer activity (D-F), for larvae (A, D), nymphs (B, E) and adults (C, F). Dotted lines represent generalised linear mixed model fit for non-significant models. Modified after T.R. Hofmeester (unpublished data).

Van Buskirk and Ostfeld (1995) modelled the relationship between host and tick density. They concluded that low densities of hosts for either adults or juveniles were insufficient to maintain populations of *Ixodes*. They also found that a change in the density of hosts for adults was accompanied by a change in tick density only at relatively low host densities. Once host densities

became high enough for all adult ticks to find a host, further increase in host density had no effect. There thus seems to be a threshold relationship rather than a linear relationship between *Ixodes* tick density and deer density, which is supported by our own data (Figure 2; T.R. Hofmeester unpublished data).

It seems likely that moderate tick densities can be sustained by already very low deer densities. A simple calculation may elucidate this idea. Assume the presence of 1 deer/km<sup>2</sup>. One deer may feed up to 500 adult females at a time (Milne 1943, Piesman *et al.* 1979, Wilson *et al.* 1990) so it will certainly do so in a season. One female produces 1,500 larvae (Hoch *et al.* 2010). If 15% of the larvae feed on a host then the next year the density of nymphs will be 11/100 m<sup>2</sup>, a moderately large tick population. We therefore agree with Van Buskirk and Ostfeld (1995) and propose the deer-tick relationship to be more of a threshold relationship than a linear one.

### Large herbivores and (effects on) habitat

Because ticks spend most of their lifetime in the vegetation rather than on a host, habitat requirements also play a key role in determining the number of ticks in an area. The persistence of ticks foremost depends on microclimate with a minimum humidity level as most important lifeline for *Ixodes ricinus* (Lees and Milne 1951). As ticks do not like to be in direct contact with water they do not occur in very wet environments like marshes but when conditions become too dry, as in many homogeneous coniferous forests or dry heathlands, conditions for tick survival decrease and tick densities become very low (Estrada-Peña 2001, Mulder 2014). Between these extremes there is a large middle ground where ticks can occur.

The humidity level is positively affected by vegetation parameters that provide shade like trees, shrubs, a tall dense herb layer and a well-developed litter layer (Dobson *et al.* 2011, Gray *et al.* 1998, Sonenshine 1991). The forest litter layer forms a refuge for ticks during unfavourable climatic conditions (Randolph and Storey 1999). The thickness of the litter layer and moss cover were positively related to nymphal and adult tick densities (Gassner *et al.* 2011). Tick density increased with shrub cover (Steigedal *et al.* 2013, Tack *et al.* 2012a, 2012b).

Together with temperature, the mentioned habitat characteristics and the composition of the host community determine the long term average tick abundance.

Figure 3 gives an impression of tick densities in different habitat types, with and without large herbivores.

In all open areas, tick densities were very low, with or without large herbivores. In our case the open areas were short grasslands or dry *Calluna* heathland with unfavourable conditions for ticks. If conditions are more favourable, when a tall or dense herb layer or shrubs are present, tick densities in open areas can be quite high, even comparable with many a woodland (James *et al.* 2013). Boyard *et al.* (2009) found an average nymph density of 27/100 m<sup>2</sup> in rough grasslands with a maximum of 388/100 m<sup>2</sup>.

Without large herbivores, ticks were present in forest habitat and in the ecotone (between the forest and the open areas) but densities were generally very low (Figure 3). The exception was an oak forest with a well-developed understory.

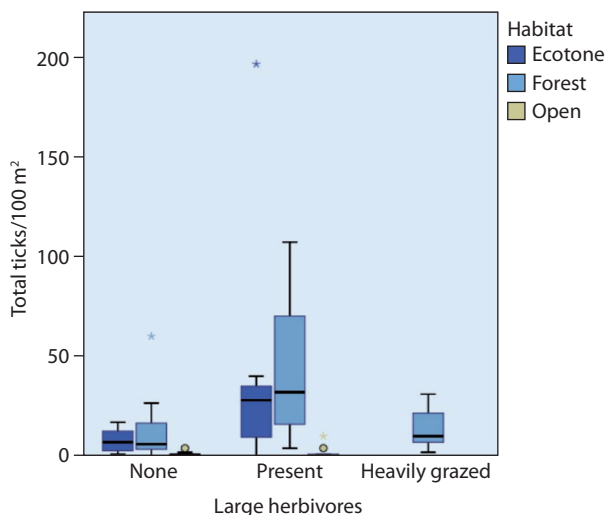


Figure 3. Boxplots of estimated tick densities (nymphs plus adults) from a number of selected habitats in the Netherlands, with and without large herbivores (45 and 31 sites, respectively), and from nine areas that were intensively grazed by sheep (2 sheep/ha).

The highest mean tick densities were found in forest habitat with large herbivores (roe deer always present, in some cases other deer species, and in a number of cases additional livestock was present at low densities) but variation was also high.

In most cases where comparisons have been made, higher tick densities have been reported in oak forests or mixed/deciduous forests compared to coniferous forests (Estrada-Peña 2001, Gray *et al.* 1998, James *et al.* 2013, Steigedal *et al.* 2013, Tack *et al.* 2012a, 2012b). Deciduous forests with a well-developed understorey most likely harbour more suitable hosts because they provide food and shelter for animals like rodents and deer (Gassner *et al.* 2008). Nevertheless coniferous forest with high rainfall and with a thick litter layer may support high tick densities too (Estrada-Peña 2001, Gray *et al.* 1998).

In general, forested habitats with a well-developed understory and a well-developed litter layer have high tick abundances because these habitats provide a suitable microclimate for tick survival and they are most likely to harbour a range of suitable hosts for ticks of all stages.

Substantial tick numbers were found in the ecotone habitats, with large herbivores, bordering the studied forests (Figure 3). From a tick-human perspective, ecotones are relevant because they are much used for recreational purposes. People use them as picnic sites, as playing ground, to put up their tent, or to walk the dog, and, as such, the ecotone is a risk habitat. High tick numbers in ecotones can, in part, be explained by ecotones still being reasonable good habitats for ticks because of the shading effects of the nearby forest. But ecotones are also preferred, or at least frequently visited, by host species. Many host species prefer forest as foraging habitat (deer) and/or as area for cover (e.g. hares, badgers, wood mice). From the forest they may go out into open areas to forage during periods of activity and spend time in the ecotone. Wood mice were more abundant in the forest-pasture ecotone than inside the forest or pastures and were thought to be

the major means of transport of *Ixodes* larvae from woodland to pasture (Boyard *et al.* 2008). Hosts may also move ticks from the woodland to adjacent open areas and so maintain tick populations there (Hoch *et al.* 2010, L'Hostis *et al.* 1995), a typical source-sink situation.

Tick densities were low in woodlands heavily grazed by, predominantly, sheep (Figure 3). All these areas were mainly heathland habitat with only small patches of woodland. The low tick numbers were obviously not because of the low density of herbivores because sheep density in these semi-natural reserves was about 2 sheep/ha. The low densities can better be explained by the alteration of the woodland through the effects of sheep grazing leaving an open forest with very little understory and a short grass layer with a thin litter layer on dry sandy soil. It is well known that large grazers can modify vegetation structure by affecting the shrub layer, preventing the development of a tree layer, and by creating a short vegetation of grasses and forbs (Van Wieren and Bakker 2008). These effects are generally observed under semi-natural conditions where domestic herbivores are held in a rangeland setting or applied for conservation purposes to mainly maintain open landscapes. In these situations, densities are higher (sometimes much higher) than the densities of wild herbivores under more natural conditions (Van Wieren and Bakker 2008). The grazing effects (under high grazing intensity) can be expected to generally affect tick populations in a negative way given the habitat requirements of ticks. Steigedal *et al.* (2013) also found lower tick densities under sheep grazing in Norway and could attribute this partly to a decrease in the shrub layer as the result of sheep grazing.

In the Netherlands large grazers like cattle and horses are sometimes applied as complement herbivores to the wild herbivores roe deer, red deer and wild boar in forest-heathland systems. Although the densities here are not as high as in the more semi-natural open landscapes, there still could be effects on tick densities through modifications of the habitat.

We compared 20, fenced, forest sites that were complementary (next to deer) grazed by cattle with 20 control sites outside the fence (Moonen 2015). The wild herbivores were also present outside the fence. We found no effects of grazing on *I. ricinus* nymphal density, herb density or saturation deficit (saturation deficit is a measure of the drying power of the atmosphere that depends on both temperature and relative humidity). We did find an effect of grazing on mean litter layer thickness ( $\beta=0.062$ ,  $SE=0.024$ ,  $P=0.009$ ; Figure 4B). The litter layer was thinner in the grazed plots.

We also found an effect on rodent burrow density (GLMM,  $\beta=-0.693$ ,  $SE=0.215$ ,  $P=0.0012$ ). Rodent burrow density was shown to be a good proxy for rodent density (Mulder 2014). Rodent density was higher in the ungrazed plots. We also found a positive relationship between rodent burrow density and *I. ricinus* nymphal density.

The relationship between herbivory and rodents is relevant because small rodents are the most important hosts for the juvenile ticks (mainly larvae) and the *Borrelia* pathogen (Hofmeester *et al.* 2016). Rodents need cover to protect them from predators and rodent density was significantly correlated with vegetation height (Smit *et al.* 2001). It is well known that grazing by large herbivores (both domestic and wild) can have negative (indirect) effects on rodent communities (Putman 1986, 1989, Smit *et al.* 2001, Van Wieren and Bakker 2008). In our study we found no effect of grazing on tick density. Apparently, nor the added grazers or the thinner litter layer, did lead to significant changes in the number of nymphal ticks produced. We did not find any changes in the herb layer.



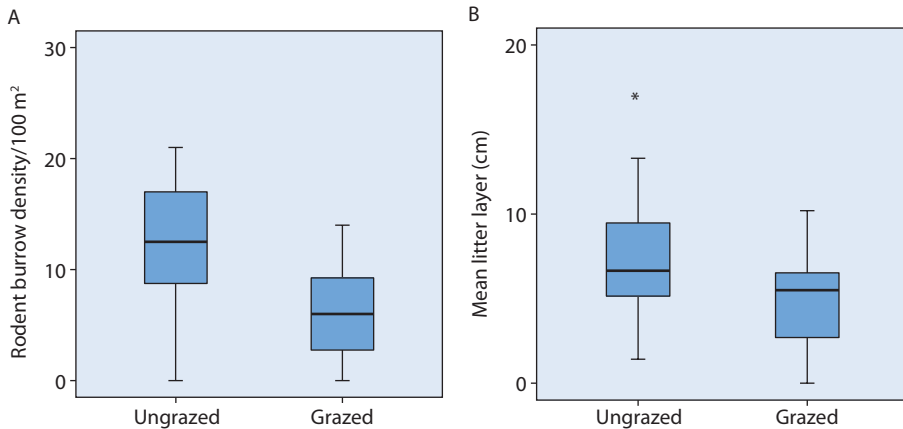


Figure 4. (A) Rodent burrow density and (B) mean thickness of the litter layer in 20 grazed and ungrazed forest sites.

It can be expected that added grazers may increase tick densities because they are additional reproduction hosts. In most cases, however, this effect will be relatively small when wild herbivores like deer are present as high tick densities are already reached at low deer densities while raised densities have never been observed. Obviously, tick numbers will increase substantially when grazers are applied in areas where no wild herbivores are present. In these cases, the interaction between the density of the grazers and their effects on the vegetation structure will determine tick density.

Grazing may have an effect on tick densities through interactions with wild herbivores. It is well known that in many situations domestic grazers compete with other wild herbivores which can lead to local habitat displacement, lower densities or even almost complete disappearance of the wild herbivores (Fritz *et al.* 1996, Kie *et al.* 1991, Osborne 1984, Putman 1986, Stewart *et al.* 2002, Van Wieren and Bakker 2008). Also Steigedal *et al.* (2013) speculate that the lower tick densities they find with sheep grazing might be partly the result of a displacement of red deer from the sheep-grazed areas. The reason for the lower tick densities, then, might be the fact that domestic animals generally have fewer ticks than deer (Porter *et al.* 2011).

### Large herbivores and *Borrelia burgdorferi* s.l.

As large herbivores are important reproduction hosts for ticks the question arises to what extent large herbivores play a role in the *Borrelia* cycle. Large herbivores can be infected with *Borrelia* but infection prevalence may vary widely (see Hofmeester *et al.* 2016). In roe deer it varied from 0-0.69 (n=8), in red deer from 0-0.45 (n=5), in fallow deer from 0.03-0.65 (n=5), in horses from 0.03-0.29 (n=7), in sheep from 0.03-0.38 (n=4), and in wild boar from 0-0.25 (n=5). Cases of Lyme borreliosis have been reported from sheep, cattle and horses (Fridriksdottir *et al.* 1992, Magnarelli *et al.* 1988, Parker and White 1992) but the disease is not known to occur in wild ungulates. In Table 2, infection prevalence of various *Borrelia* species are given that have been found in engorged ticks taken from a number of ungulate species in European systems.

The infection prevalence is generally very low. In fact, it has always been found to be much lower than the prevalence in questing ticks in the vegetation (Table 3). This suggests that large herbivores

Table 2. Infection prevalence for various species of *Borrelia* in engorged ticks taken from large herbivores.<sup>1,2</sup>

Host species	Pathogen	Hosts tested	Total ticks tested	Infection prevalence				Reference
				All ticks	Larvae	Nymphs	Adults	
moose	<i>B. afzelii</i>	52	685	0.03	0.00	0.03	0.05	1
moose	<i>B. burgdorferi</i> s.s.	52	685	0.00	0.00	0.00	0.00	1
moose	<i>B. garinii</i>	52	685	0.01	0.00	0.01	0.01	1
moose	<i>B. burgdorferi</i> s.l.	7	126	0.00	0.00	ND	ND	2
roe deer	<i>B. burgdorferi</i> s.l.	16	238	0.00	0.00	ND	ND	3
roe deer	<i>B. burgdorferi</i> s.s.	18	934	0.00	0.00	0.00	0.00	1
roe deer	<i>B. afzelii</i>	18	934	0.01	0.00	0.02	0.00	1
roe deer	<i>B. garinii</i>	18	934	0.00	0.00	0.01	0.00	1
roe deer	<i>B. burgdorferi</i> s.l.	16	11	0.09	ND	ND	ND	4
roe deer	<i>B. afzelii</i>	ND	190	0.15	ND	ND	ND	5
roe deer	<i>B. burgdorferi</i> s.l.	47	106	0.23	ND	ND	0.23	5
roe deer	<i>B. burgdorferi</i> s.s.	ND	190	0.01	ND	ND	ND	5
roe deer	<i>B. garinii</i>	ND	190	0.01	ND	ND	ND	5
roe deer	<i>B. valaisiana</i>	ND	190	0.02	ND	ND	ND	5
roe deer	coinfection <i>B. afzelii</i> + <i>B. burgdorferi</i> s.s.	ND	190	0.03	ND	ND	ND	5
roe deer	coinfection <i>B. afzelii</i> + <i>B. garinii</i>	ND	190	0.01	ND	ND	ND	5
roe deer	coinfection <i>B. afzelii</i> + <i>B. valaisiana</i>	ND	190	0.04	ND	ND	ND	5
roe deer	<i>B. afzelii</i>	ND	121	0.07	ND	0.09	0.07	6
roe deer	<i>B. burgdorferi</i> s.l.	ND	121	0.13	ND	0.09	0.14	6
roe deer	<i>B. valaisiana</i>	ND	121	0.02	ND	0.00	0.03	6
roe deer	coinfection <i>B. afzelii</i> + <i>B. garinii</i>	ND	121	0.01	ND	0.00	0.01	6
roe deer	coinfection <i>B. afzelii</i> + <i>B. valaisiana</i>	ND	121	0.02	ND	0.00	0.02	6
roe deer	coinfection <i>B. burgdorferi</i> s.s. + <i>B. garinii</i>	ND	121	0.01	ND	0.00	0.01	6
roe deer	<i>B. burgdorferi</i> s.l.	20	44	0.09	ND	ND	0.09	7
red deer	<i>B. burgdorferi</i> s.l.	48	67	0.01	ND	ND	ND	4
red deer	<i>B. burgdorferi</i> s.l.	12	195	0.01	ND	ND	0.01	7
fallow deer	<i>B. burgdorferi</i> s.l.	38	86	0.01	ND	ND	ND	4
horse	<i>B. afzelii</i>	ND	7	0.43	ND	ND	ND	8
sheep	<i>B. burgdorferi</i> s.l.	ND	347	0.18	0.07	0.17	0.25	9
mouflon	<i>B. burgdorferi</i> s.l.	29	33	0.03	ND	ND	ND	4
wild boar	<i>B. burgdorferi</i> s.l.	1	7	0.29	ND	ND	0.29	7

<sup>1</sup> References: 1 = Kjelland *et al.* (2011); 2 = Tälleklint and Jaenson (1994); 3 = Jaenson and Tälleklint (1992); 4 = Matuschka *et al.* (1993); 5 = Rijpkema *et al.* (1996); 6 = Schouls *et al.* (1999); 7 = Wegner *et al.* (1997); 8 = Ionita *et al.* (2013); 9 = Ogden *et al.* (1997).

<sup>2</sup> ND = not determined.

Table 3. Summary overview of reported infection prevalence of *Borrelia burgdorferi* s.l. in engorged ticks (nymphs and/or adults) taken from large herbivores and from questing ticks in the vegetation.

Species	Prevalence engorged ticks	Prevalence questing ticks	Reference
roe, red, fallow deer	0.03	0.17	Matuschka <i>et al.</i> (1993)
goats, cattle	0.01	0.23	Richter and Matuschka (2010)
red deer, wild boar	0.01	0.09	Pacilly <i>et al.</i> (2014)
sheep	0.01	0.10	this study

are not very competent in transmitting the bacterium to the ticks and there is even evidence that the complement of these species might clear the infection from feeding ticks (Kurtenbach *et al.* 2002). In a series of *in vitro* experiments, Kurtenbach *et al.* (2002) also found that competence of domestic sheep, cattle and horses was not total for *Borrelia burgdorferi* s.l., although it was for some other species of *Borrelia*.

If large herbivores are largely incompetent for transmitting *Borrelia* to ticks and even may clear the infection from ticks that feed on them, the question can be raised what role herbivores play in the *Borrelia* s.l. cycle.

It is likely that ticks that are added to the system through large herbivores will not be infected. If questing larvae, that have a very low infection prevalence (Van Duijvendijk *et al.* 2016), will feed on a large herbivore, the nymphs in the next stage will not be infected. Larvae generally get infected when feeding on a competent infected host and these are mainly smaller species, notably rodents. Although large herbivores feed most of the adult ticks and juvenile ticks mainly feed on small species, large herbivores nevertheless sometimes carry many larvae as well. At very high herbivore densities a dilution effect might then become apparent. Very little work has been done to study the effects of varying large herbivore densities on the density of infected nymphs (DIN), the most relevant risk factor for humans. James *et al.* (2013) report higher infection prevalence in areas with higher deer abundance. They also found higher infection prevalence in mixed/deciduous forests when compared to coniferous forests. In contrast, Mysterud *et al.* (2016) found a negative correlation between deer density and infection prevalence with *B. burgdorferi* s.l. in questing nymphs. However, it is not always straightforward how these differences in infection prevalence affect the DIN.

Richter and Matuschka (2006) found a negative effect of cattle on *B. burgdorferi* prevalence within French pastures. In the study mentioned above, where we compared 20 grazed (with cattle) forest sites with 20 ungrazed control sites, we found no effects of grazing on *B. burgdorferi* prevalence in nymphs and adults of *I. ricinus*. The value of the results of all of these studies is very limited because the effects of important other hosts have not been taken into account. In particular rodents should be included in any such study. The density of infected nymphs is the result of the interplay between the densities of all relevant hosts, their capacity to feed ticks and their capacity to transmit the bacteria to the ticks (Hofmeester *et al.* 2016, see also Van Wieren, 2016).

It may be clear that large herbivores play a key role in maintaining tick populations in most European systems because they are the most important reproduction hosts. The relationship

between herbivore abundance and tick density and infection prevalence with *B. burgdorferi*, however, varies between countries. In the past decades tick densities have increased greatly together with an increase in distribution, both latitudinally and altitudinally, due to changes in habitat characteristics and host densities (Medlock *et al.* 2013). Important drivers for this change are increasing temperatures, an increase in forest cover, changes in forest management, a higher frequency of mast years, range expansion of roe deer, red deer and wild boar, a lower hunting pressure on these species, changes in agricultural use (land abandonment, bush encroachment), agricultural schemes, and increased connectivity between habitats (Apollonio *et al.* 2010). In many systems a large number of these factors act simultaneously and in conjunction because they all have a positive effect on tick populations.

### Public health relevance

- Large herbivores are the most important species for maintaining tick populations.
- At high density large herbivores can manipulate the vegetation to such an extent that survival conditions for ticks are being compromised but also that exposure of ticks to humans is reduced.
- The density of infected nymphs, being the greatest risk factor for humans, is not likely to be much affected by varying herbivore density, except when this density is almost zero, or when densities are exceptionally high.

### References

- Apollonio M, Andersen R and Putman R (2010) European ungulates and their management in the 21<sup>st</sup> century. Cambridge University Press, Cambridge, UK.
- Boyard C, Vourc'h G and Barnouin J (2008) The relationships between *Ixodes ricinus* and small mammal species at the woodland-pasture interface. *Exp Appl Acarol* 44: 61-76.
- Dobson ADM, Taylor JL and Randolph SE (2011) Tick (*Ixodes ricinus*) abundance and seasonality at recreational sites in the UK: hazards in relation to fine-scale habitat types revealed by complementary sampling methods. *Ticks Tick Borne Dis* 2: 67-74.
- Domínguez-Peñafiel G, Giménez-Pardo C, Gegúndez MI and Lledó L (2011) Prevalence of ectoparasitic arthropods on wild animals and cattle in the Las Merindades area (Burgos, Spain). *Parasite* 18: 251-260.
- Estrada-Peña A (2001) Distribution, abundance, and habitat preferences of *Ixodes ricinus* (acari: Ixodidae) in northern Spain. *J Med Entomol* 38: 361-370.
- Fridriksdottir V, Nesse LL and Gudging R (1992) Seroepidemiological studies of *Borrelia burgdorferi* infection in sheep in Norway. *J Clin Microbiol* 30: 1271-1277.
- Fritz RS, Roche BM, Brunsfeld SJ and Orians CM (1996) Interspecific and temporal variation in herbivore responses to hybrid willows. *Oecologia* 108: 121-129.
- Gassner F, van Vliet AJH, Burgers S, Jacobs F, Verbaarschot P, Hovius EKE, Mulder S, Verhulst NO, van Overbeek LS and Takken W (2011) Geographic and temporal variations in population dynamics of *Ixodes ricinus* and associated *Borrelia* infections in the Netherlands. *Vector Borne Zoonotic Dis* 11: 523-532.
- Gassner F, Verbaarschot P, Smallegange RC, Spitzen J, Van Wieren SE and Takken W (2008) Variations in *Ixodes ricinus* density and *Borrelia* infections associated with cattle introduced into a woodland in the Netherlands. *Appl Environ Microbiol* 74: 7138-7144.

- Gray JS, Kahl O, Robertson JN, Daniel M, Estrada-Pena A, Gettinby G, Jaenson TGT, Jensen P, Jongejan F, Korenberg E, Kurtenbach K and Zeman P (1998) Lyme borreliosis habitat assessment. *Zentralbl Bakteriol* 287: 211-228.
- Handeland K, Qviller L, Vikøren T, Viljugrein H, Lillehaug A and Davidson RK (2013) *Ixodes ricinus* infestation in free-ranging cervids in Norway – a study based upon ear examinations of hunted animals. *Vet Parasitol* 195: 142-149.
- Heyl J and de Mendonca PG (2011) Tick infestation in roe deer (*Capreolus capreolus*) from Thuringia (Germany). *Acta Zoologica Bulgarica* 63: 313-317.
- Hoch T, Monnet Y and Agoulon A (2010) Influence of host migration between woodland and pasture on the population dynamics of the tick *Ixodes ricinus*: a modelling approach. *Ecol Model* 221: 1798-1806.
- Hofmeester TR, Coipan EC, Van Wieren SE, Prins HHT, Takken W and Sprong H (2016) Few vertebrate species dominate the *Borrelia burgdorferi* s.l. life cycle. *Environ Res Lett* 11: 043001.
- Hornok S, Horváth G, Jongejan F and Farkas R (2012) Ixodid ticks on ruminants, with on-host initiated moulting (apolysis) of *Ixodes*, *Haemaphysalis* and *Dermacentor* larvae. *Vet Parasitol* 187: 350-353.
- Ionita M, Mitrea IL, Pfister K, Hamel D and Silaghi C (2013) Molecular evidence for bacterial and protozoan pathogens in hard ticks from Romania. *Vet Parasitol* 196: 71-76.
- Jaenson TGT and Tälleklint L (1992) Incompetence of roe deer as reservoirs of the Lyme borreliosis spirochete. *J Med Entomol* 29: 813-817.
- James MC, Bowman AS, Forbes KJ, Lewis F, McLeod JE and Gilbert L (2013) Environmental determinants of *Ixodes ricinus* ticks and the incidence of *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. *Parasitology* 140: 237-246.
- Jensen, PM and Jespersen JB (2005) Five decades of tick-man interaction in Denmark – an analysis. *Exp Appl Acarol* 35: 131-146.
- Kie JG, Evans CJ, Loft ER and Menke JW (1991) Foraging behavior by mule deer: the influence of cattle grazing. *J Wildl Manage* 55: 665-674.
- Kiffner C, Lodge C, Alings M, Vor T and Ruhe F (2010) Abundance estimation of *Ixodes* ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). *Exp Appl Acarol* 52: 73-84.
- Kjelland V, Ytrehus B, Stuen S, Skarpaas T and Slettan A (2011) Prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* ticks collected from moose (*Alces alces*) and roe deer (*Capreolus capreolus*) in southern Norway. *Ticks Tick Borne Dis* 2: 99-103.
- Kurtenbach K, De Michelis S, Etti S, Schafer SM, Sewell HS, Brade V and Kraiczky P (2002) Host association of *Borrelia burgdorferi* sensu lato – the key role of host complement. *Trends Microbiol* 10: 74-79.
- L'Hostis M, Diarra O and Seegers H (1994) Sites of attachment and density assessment of female *Ixodes ricinus* (Acari: Ixodidae) on dairy cows. *Exp Appl Acarol* 18: 681-689.
- L'Hostis M, Dumon H, Dorchies B, Boisdrion F and Gorenflot A (1995) Seasonal incidence and ecology of the tick *Ixodes ricinus* (Acari: Ixodidae) on grazing pastures in western France. *Exp Appl Acarol* 19: 211-220.
- Lees AD and Milne A (1951) The seasonal and diurnal activities of individual sheep ticks (*Ixodes ricinus* L.). *Parasitology* 41: 189-208.
- Magnarelli LA, Anderson JF, Shaw E, Post JE and Palka FC (1988) Borreliosis in equids in northeastern United States. *Am J Vet Res* 49: 359-362.
- Matuschka FR, Heiler M, Eiffert H, Fischer P, Lotter H and Spielman A (1993) Diversionary role of hoofed game in the transmission of Lyme disease spirochetes. *Am J Trop Med Hyg* 48: 693-699.
- Medlock JM, Hansford KM, Bormane A, Derdakova M, Estrada-Peña A, George JC, Golovljova I, Jaenson TGT, Jensen JK, Jensen PM, Kazimirova M, Oteo JA, Papa A, Pfister K, Plantard O, Randolph SE, Rizzoli A, Santos-Silva MM, Sprong H, Vial L, Hendrickx G, Zeller H and Van Bortel W (2013) Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. *Parasit Vectors* 6: 1.
- Milne A (1943) The comparison of sheep-tick populations (*Ixodes ricinus* L.). *Ann Appl Biol* 30: 240-250.
- Milne A (1947) The ecology of the sheep tick, *Ixodes ricinus* L. The infestations of hill sheep. *Parasitology* 38: 34-50.
- Moonen S (2015) Effect of cattle grazing on *Ixodes ricinus* and the prevalence of *Borrelia burgdorferi* s.l. MSc thesis, Wageningen University & Research, Wageningen, the Netherlands.

- Mulder AC (2014) Deer and rabbit density as determinants of tick density on a local scale. MSc thesis, Wageningen University & Research, Wageningen, the Netherlands.
- Mysterud A, Easterday WR, Stigum VM, Aas AB, Meisingset EL and Viljugrein H (2016) Contrasting emergence of lyme disease across ecosystems. *Nat Commun* 7: 11882.
- Ogden NH, Nuttall PA and Randolph SE (1997) Natural Lyme disease cycles maintained via sheep by co-feeding ticks. *Parasitology* 115: 591-599.
- Osborne BC (1984) Habitat use by red deer (*Cervus elaphus* L.) and hill sheep in the west Highlands. *J Appl Ecol* 21: 497-506.
- Pacilly FCA, Benning ME, Jacobs F, Leidekker J, Sprong H, Van Wieren SE and Takken W (2014) Blood feeding on large grazers affects the transmission of *Borrelia burgdorferi* sensu lato by *Ixodes ricinus*. *Ticks Tick Borne Dis* 5: 810-817.
- Parker JL and White KK (1992) Lyme borreliosis in cattle and horses: a review of the literature. *Cornell Vet* 82: 253-274.
- Pato FJ, Panadero R, Vázquez L, López CM, Díaz P, Vázquez E, Díez-Baños P, Morrondo P and Fernández G (2013) Seroprevalence of *Borrelia burgdorferi* sensu lato in roe deer (*Capreolus capreolus*) from northwestern Spain. *J Zoo Wildl Med* 44: 660-665.
- Piesman J, Spielman A, Etkind P, Ruebush TK and Juranek DD (1979) Role of deer in the epizootiology of *Babesia microti* in Massachusetts, USA. *J Med Entomol* 15: 537-540.
- Porter R, Norman R and Gilbert L (2011) Controlling tick-borne diseases through domestic animal management: a theoretical approach. *Theor Ecol* 4: 321-339.
- Putman RJ (1986) Competition and coexistence in a multi-species grazing system. *Acta Theriol* 31: 271-291.
- Putman RJ, Edwards PJ, Mann JCE, How RC and Hill SD (1989) Vegetational and faunal changes in an area of heavily grazed woodland following relief of grazing. *Biol Conserv* 47: 13-32.
- Rand PW, Lubelczyk C, Lavigne GR, Elias S, Holman MS, Lacombe EH and Smith RP (2003). Deer density and the abundance of *Ixodes scapularis* (Acari: Ixodidae). *J Med Entomol* 40: 179-184.
- Randolph SE and Storey K (1999) Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. *J Med Entomol* 36: 741-748.
- Richter D and Matuschka FR (2006) Modulatory effect of cattle on risk for Lyme disease. *Emerging Infect Dis* 12: 1919-1923.
- Richter D and Matuschka FR (2010) Elimination of Lyme disease spirochetes from ticks feeding on domestic ruminants. *Appl Environ Microbiol* 76: 7650-7652.
- Rijpkema SGT, Herbes RG, Verbeek de Kruijff N and Schellekens JFP (1996) Detection of four species of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected from roe deer (*Capreolus capreolus*) in the Netherlands. *Epidemiol Infect* 117: 563-566.
- Schouls LM, Van De Pol I, Rijpkema SGT and Schot CS (1999) Detection and identification of ehrlichia, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in dutch *Ixodes ricinus* ticks. *J Clin Microbiol* 37: 2215-2222.
- Smit R, Bokdam J, den Ouden J, Olff H, Schot-Opschoor H and Schrijvers M (2001) Effects of introduction and exclusion of large herbivores on small rodent communities. *Plant Ecol* 155: 119-127.
- Sonenshine DE (1991) *Biology of ticks*. Oxford University Press, New York, NY, USA.
- Sprong H, Hoffhuis A, Gassner F, Takken W, Jacobs F, Van Vliet AJH, Van Ballegooijen M, Van der Giessen J and Takumi K (2012) Circumstantial evidence for an increase in the total number and activity of *Borrelia*-infected *Ixodes ricinus* in the Netherlands. *Parasit Vectors* 5: 1.
- Steigedal HH, Loe LE, Grøva L and Mysterud A (2013) The effect of sheep (*Ovis aries*) presence on the abundance of ticks (*Ixodes ricinus*). *Acta Agric Scand A Anim Sci* 63: 111-120.
- Stewart KM, Bowyer RT, Kie JG, Cimon NJ and Johnson BK (2002) Temporospatial distributions of elk, mule deer, and cattle: resource partitioning and competitive displacement. *J Mammal* 83: 229-244.
- Tack W, Madder M, Baeten L, De Frenne P and Verheyen K (2012a) The abundance of *Ixodes ricinus* ticks depends on tree species composition and shrub cover. *Parasitology* 139: 1273-1281.
- Tack W, Madder M, Baeten L, Vanhellefont M, Gruwez R and Verheyen K (2012b) Local habitat and landscape affect *Ixodes ricinus* tick abundances in forests on poor, sandy soils. *For Ecol Manage* 265: 30-36.

- Tälleklint L and Jaenson TGT (1994) Transmission of *Borrelia burgdorferi* s.l. from mammal reservoirs to the primary vector of lyme borreliosis, *Ixodes ricinus* (Acari: Ixodidae), in Sweden. *J Med Entomol* 31: 880-886.
- Tälleklint L and Jaenson TGT (1997) Infestation of mammals by *Ixodes ricinus* ticks (Acari: Ixodidae) in south-central Sweden. *Exp Appl Acarol* 21: 755-771.
- Van Buskirk J and Ostfeld RS (1995) Controlling Lyme disease by modifying the density and species composition of tick hosts. *Ecol Appl* 5: 1133-1140.
- Van Duijvendijk G, Coipan C, Wagemakers A, Fonville M, Ersöz J, Oei A, Földvári G, Hovius J, Takken W and Sprong H (2016) Larvae of *Ixodes ricinus* transmit *Borrelia afzelii* and *B. miyamotoi* to vertebrate hosts. *Parasit Vectors* 9: 97.
- Van Wieren SE (2016) Sheep mopping. In: Braks MAH, Van Wieren SE, Takken W and Sprong H (eds.) Ecology and prevention of Lyme borreliosis. Ecology and Control of Vector-borne diseases, Volume 4. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 253-263.
- Van Wieren SE and Bakker JP (2008) The impact of browsing and grazing herbivores on biodiversity. In: Gordon IJ and Prins HHT (eds.) The ecology of browsing and grazing. Springer, Berlin/Heidelberg Germany, pp. 263-292.
- Vazquez L, Panadero R, Dacal V, Pato FJ, Lopez C, Diaz P, Arias MS, Fernandez G, Diez-Baos P and Morrondo P (2011) Tick infestation (Acari: Ixodidae) in roe deer (*Capreolus capreolus*) from northwestern Spain: population dynamics and risk stratification. *Exp Appl Acarol* 53: 399-409.
- Vor T, Kiffner C, Hagedorn P, Niedrig M and Ruhe F (2010) Tick burdens on european roe deer (*Capreolus capreolus*). *Exp Appl Acarol* 51: 405-417.
- Wegner Z, Stańczak J, Racewicz M, Kubica-Biernat B and Kruminis-Łozowska W (1997) The etiological agent of Lyme disease, *Borrelia burgdorferi*, in ticks (Acari: Ixodidae) from eastern Poland. *Zentralbl Bakteriologie* 286: 93-106.
- Wilson ML, Ducey AM, Litwin TS, Gavin TA and Spielman A (1990) Microgeographic distribution of immature *Ixodes dammini* ticks correlated with that of deer. *Med Vet Entomol* 4: 151-159.