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Master thesis

The role of cervids and wild boar in the prevalence of tick-borne encephalitis in Sweden

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Abstract

Roe deer have been historically considered the main large mammal host for *Ixodes ricinus* ticks and its population levels have been related to the current distribution and prevalence of tick-borne encephalitis virus (TBEv) in Sweden. It was thought that the increases in this disease were due to the roe deer population peak and a series of mild winters in the nineties. The roe deer is now at levels similar to the seventies, but the incidence of both TBEv and ticks are increasing. The role Swedish Association for Hunting and Wildlife Management, 2013 that other large mammal species play on this could be important to understand the current situation. We analysed the presence or absence of tick-borne encephalitis virus antibodies on blood samples from 259 individuals of fallow deer, moose, red deer, roe deer and wild boar from blood samples collected by hunters during the hunting seasons of 2010 to 2013 in 31 municipalities in south-central and southern Sweden. We found antibodies for tick-borne encephalitis in all five species, with the highest prevalence in roe deer (50% of positives), followed by moose (42%), red deer (42%), wild boar (32%) and fallow deer (25%). The prevalence was not significantly different between the species. However, age and sex significantly affected the probability of infection.

This study demonstrates that not only roe deer, but the other four species analysed are important hosts for tick-borne encephalitis virus. The increase of these populations may cause an increase of *Ixodes ricinus* population and the incidence of TBEv, and they should be taken into account in the management of the wild populations to prevent an increase in human infections.

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Introduction

For several decades, roe deer (*Capreolus capreolus)* have been considered to be the primary host for *Ixodes ricinus* ticks in Sweden, acting as the most important blood meal for female ticks and mate-seeking site for tick males (Jaenson et al, 2012,a).

Ixodes ricinus is the most common tick in Sweden and the rest of Europe, causing 90-95% of all tick bites in humans (Suss et al. 2008). This tick species plays an important role in the spread of tick-borne encephalitis virus (TBEv), the most common vector-borne virus in Europe (Jaenson et al. 2009). TBEv is a *flavivirus* from the family *flaviviridae* with three subtypes: Western, Siberian and Far East, the former being transmitted by *Ixodes ricinus*, and the others, by *Ixodes persulcatus* (Roelandt et al. 2010). The life cycle of *Ixodes ricinus* lasts around two years, and consists of three life stages (larvae, nymph and adult), feeding once per stage and in different hosts. The tick-to-tick transmission of pathogens can be transstadially, horizontally and trans-ovarially (Roelandt et al. 2010). Horizontal transmission occurs when the ticks form feeding clusters on specific parts of the host, and this proximity promotes virus transmission from infected to uninfected ticks (Randolph et al. 1996). The three life stages can bite humans, but the most important stage in the viral transmission to humans, are the nymphs, who are the more abundant, smaller and more difficult to detect than adults (Salman & Tarrés-Call, 2013). The prevalence of TBEv in the *Ixodes ricinus* population seems to be quite low. Pettersson et al. (2014) calculated a general minimum infection rate (MIR) of 0.10% for nymphs, and 0.55% for adult females. However, they suggest that this calculation may underestimate the actual MIR. Overall, they estimated that one of 360 ticks in Scandinavia is positive for TBEv.

This tick species feed on a broad spectrum of domestic and wild species, mainly mammals but also birds and reptiles (Suss, 2003). Other species of cervids including fallow deer (*Dama dama*), red deer (*Cervus elaphus*) and moose (*Alces alces*), predators as red fox (*Vulpes vulpes*) and wild boar (*Sus scrofa*) are common blood sources (Cisak et al., 2012, Handeland et al., 2013, Jemersic et al., 2014,). Larva and nymph stages may feed on small hosts including rodents and large hosts such as deer, but the adult ticks feed mainly on larger hosts (Medlock et al. 2013).

A study carried out in Eastern Poland by Cisak et al (2012) suggests that wild boar, rather than roe deer, plays the most important role in the prevalence of TBE in endemic areas. In Sweden, wild boar was part of the native fauna until it became extinct in the sixteenth century (Lemel et al., 2003), but in 1970s, individuals that escaped or were released from captivity spread and established sustainable populations (Salman & Tarrés-Call, 2013). According to the harvest statistics from the Swedish Association for Hunting and Wildlife management, more than 97,000 wild boars were shot in 2012 (Jonas Kindberg, personal notification). Therefore, there is a large wild boar population widely distributed at local and regional level in southern and south-central Sweden.

Moose are described as an important tick host in North America, where each individual can carry over 50,000 winter ticks (*Dermacentor albipictus*) (Franzmann & Schwartz, 2007). A study carried out in Norway where ears from moose, red deer and roe deer were collected and analysed for ticks concluded that the three species are heavily infested with ticks, although the level was lower in moose than in the other two deer species (Handeland et al. 2013). In Sweden, the moose population started growing significantly from the mid-seventies, reaching a peak in 1982, when 183,000 moose were shot (Apollonio et al. 2010). The population decreased in the next decade but is now, according to harvest statistics (from 81,000 in 2007 to 96,000 in 2012) increasing again and is spread all over the country. (Jonas Kindberg, personal notification).

Red deer population remained at very low levels in Sweden until the seventies, when it started growing to current levels, an estimated 10,000 animals (Apollonio et al. 2010). As is written in the paragraph above, Handeland et al (2013) found high level of tick infestation in red deer similar to the level in roe deer, thus this species, although not widely distributed, could play an important role in the maintenance of tick populations in areas where red deer are prevalent.

The fallow deer population has also increased substantially since the beginning of the 1990s (Apollonio et al. 2010). However, the population is relatively low, in comparison to moose and roe deer populations, with just over 31,000 the fallow deer shot in 2012 (Jonas Kindberg, personal notification).

The roe deer population reached one million in Sweden in the nineties mainly due to sarcoptic mange affecting the red fox and european lynx (*Lynx lynx*) populations, the main predators for roe deer, during the 1970s and1980s and the mild winters in the early 1990s (Lindstrom et al., 1994, Jaenson, 2012,a). This population began declining with the increase of red fox and lynx, and after the harsh winters of 2009/10 and 2010/11, the roe deer population has suffered a hard decline (Jaenson et al. 2012a). Thus, in the hunting season of 2012, approximately 96,000 roe deer were harvested, similar amount to the moose harvested in the same year (Jonas Kindberg, personal notification).

This roe deer explosion and the mild winters in the nineties are the main arguments used to explain the *Ixodes ricinus* ticks population growth and expansion further north along the eastern coast (Jaenson et al. 2012,a). Today, despite the roe deer population decreasing to levels similar to the late 1970s (Apollonio et al. 2010), ticks continue to be abundant throughout their range, from the Finish border in the northern part of the Gulf of Bothnia, and the cases of TBE infections continue increasing with 284 cases in 2011 and 287 in 2012, the highest incidence ever recorded in Sweden (Swedish Institute for Infectious Disease Control, 2014). Thus, the roe deer abundance as the most important factor to explain the distribution and abundance of *Ixodes ricinus* may be not adequately explain the current TBEv distribution. Furthermore, nowadays wild boar is a “new” blood source spreading quickly throughout central and southern Sweden, and the other cervid populations have grown significantly in the last decades (Jonas Kindberg, personal notification).

In this study, we analyse the presence or absence of TBEv infection in moose, red deer, roe deer, fallow deer and wild boar harvested during ordinary hunts, to better describe the role of these five large mammal species in the distribution of tick-borne encephalitis virus. The followin questions are addressed:

1. Do moose, red deer, roe deer, fallow deer and wild boar have a similar prevalence of TBEv infection?
2. Does gender and/or age influence on the probability of TBEv infection?

Material and methods

Study area

The study was performed at several places in southern Sweden (from 60ºN to the southern coast, except for Holmön Island, in Umeå, which was 63º43'N 20º51'E, see Figure 1A). The southern region is historically the most affected by TBE in Sweden, and including the northern island of Holmön allowed more overlap with the *Ixodes ricinus* tick distribution. Currently the *Ixodes ricinus* range extends throughout the east coast. However, more cases of TBE infection are registered south of 60ºN, with the most affected counties being Stockholm, Södermanland and Uppland (Jaenson et al. 2012b). About 60ºN there is a biogeographical boundary called "*Limes* Norrlandicus". This boundary crosses Sweden from the south extreme border with Norway in the west, to the west coast in the north limit of the Uppland County (Cultbase, Pan Project). The biogeographical region under *Limes Norrlandicus* is called Nemoral zone, and is composed by mixed forest, and commons species are *Picea abies, Alnus incana, Alnus glutinosa, Ulmus glabra, Fraxinus excelsior, Tilia cordata* and *Corylus avellana.* It is also a region rich in fauna, being the general pattern a negative gradient in the richness from south to north. Nemoral zone present also a significant change of monthly mean temperatures and duration of snow cover (Cultbase, Pan Project). South and South-Central Sweden are regions rich in water masses, with the biggest lakes of the country. All of these factors (more temperate climate, water availability and fauna and flora richness) are important factors in the distribution and prevalence of *Ixodes ricinus* and TBEv.

Blood sampling

The blood samples were obtained through the years 2010 to 2013 by hunters in several municipalities of 10 counties (Table 1), during the hunting seasons for each species. The technique to collect the blood samples was by blood absorption on Whatman Grade 3MM Chr Cellulose Paper (GE Healthcare UK Ltd. Buckinghamshire, England). Each filter-paper was soaked with blood, in the woods or at a facility for slaughtering, ensuring no contamination between specimens. The blood soaked paper was subsequently dried in room temperature before posted by surface mail to the Swedish University of Agricultural Science

(SLU, Umeå) in individual plastic ZIP-lock bags. This sampling method is very convenient, allowing for handling and storing at room temperature, being possible to be carried out appropriately by hunters (see Appendix).

Table 1. Number of samples collected for each species and County.

	Fallow deer	Moose	Red deer	Roe deer	Wild boar	TOTAL
Halland					15	15
Jönköping		1			2	3
Kalmar					1	1
Kronoberg				2	14	16
Skåne	1		1	6	11	19
Stockholm		6		5	29	62
Södermanland	59	9	23	8	44	121
Uppland				1	4	5
Vasterbotten		14				14
Västra Götaland					2	2
Unknown		1				1
TOTAL	60	31	24	22	122	**259**

Blood elution and Antibody detection

Following instructions on using Nobuto blood filter strips (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), approximately 1 cm^2 was cut from each filter-paper in two-three smaller pieces and put in an tube 2 ml eppendorf with 1 ml of Phosphate buffered saline (PBS). After 1 hour, the filter-paper pieces were removed and the eppendorf tubes kept at -20 ºC until testing.

The determination of antibody against TBEv was made by The Immunozym FSME IgG All Species-ELISA® (PROGEN Biotechnik, Heidelberg, Germany) following the protocol of the package insert. The amount of immonoglobins against TBEv detected was measured in Vienna Units per millilitre (VIEU/ml), sera from 63 VIEU/ml to 126 VIEU/ml were considered borderline, sera with concentrations lower than 63 VIEU/ml as negative and sera with a concentration higher than 126 VIEU/ml as positive.

Data analysis

To test if there are significant differences in prevalence between the different species, I used R (R Development Core Team, Austria, 2008) to build a general linear model with species as an independent variable and the presence or absence of antibodies as a response variable, and performing an ANOVA test afterwards. I did this at different scales, narrowing the analysis from the “whole” country to a local view. I also tested gender and age as factors having an influence on the probability of infection in each species. To do that, I compared five general linear mixed models including all of the possible combinations of the variables (Table 2). Due to the circumstances that the samples come from different places, and the geographically patchy distribution of TBEv in the environment, the individual specimens may experience different probabilities of TBEv infection that may be affected by gender and age factors. I corrected for this “risk” by adding the variable municipality as a fixed factor. I calculated the corrected Akaike´s information criteria (AICc), ΔAICc and AICc in order to estimate which of the models fit the data best. I considered models with ΔAICc lower than 2 to be equally valid. (Bolker et al. 2009)

Table 2. List of the models built for each species. The names are used for reference.

Names	Model
Full	Gender+Age+Gender*Age+(1|Municipality)
Gender,Age	Gender+Age+(1|Municipality)
Gender	Gender+(1|Municipality)
Age	Age+(1|Municipality)
Null	1+(1|Municipality)

Results

Antibodies prevalence

In all, 88 of the 259 blood samples were positive for TBEv antibodies, representing 9 of the 28 municipalities where samples were collected (Table 3, Figure 1B). All of the municipalities with positive samples are located in south and south-central Sweden except for Umeå in northern Sweden. Samples from all five species were found positive, with the highest prevalence in roe deer (50.0%), follow by moose (41.9%), red deer (41.7%), wild boar (32.0%), and finally fallow deer (25.0%).

In general, there were no significant differences in prevalence between species ($X^2_{4,254}$ = 6.33, P = 0.17), varying between 30-50%, and the overall TBEv prevalence in this study is 34%. In order to have a narrow view, I analysed the samples from Södermanland County

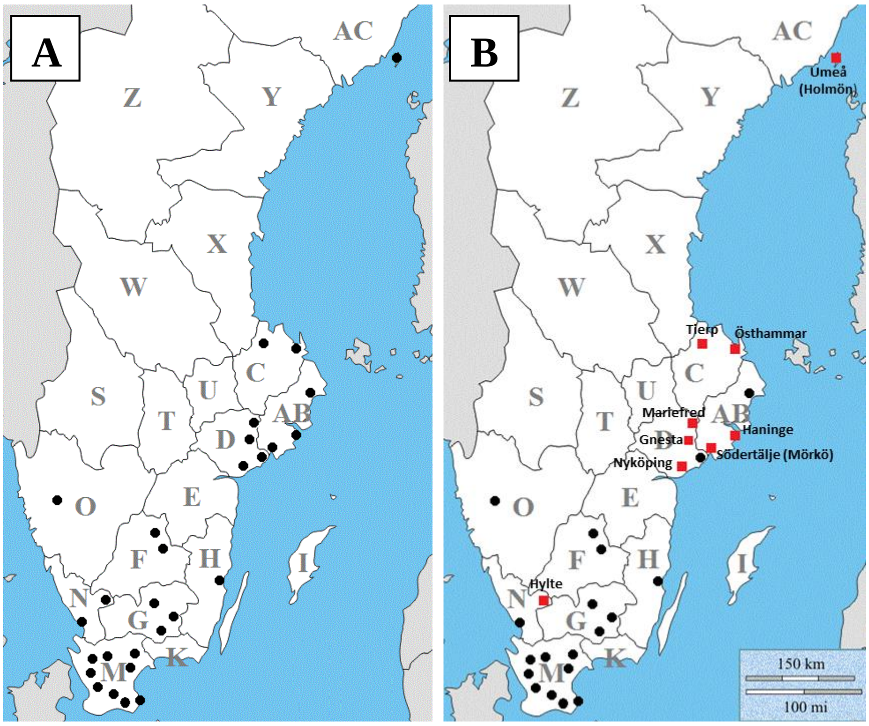

Figure 1. Partial map of Sweden showing all the sampling sites (black dots in map A) and the places where samples were positive for TBE antibodies (Red squares in map B). The counties sampled were Vasterbotten (AC), Uppland (C), Stockholm (AB), Södermanland (D), Västra Götaland, Jönköping (F), Kalmar (H), Halland (N), Kronoberg (G) and Skåne (M).

where the 55% of all samples were collected. The prevalence here was quite different, being a 66.7% for moose, 62.5% for roe deer, 43.5% for red deer, 34,1% for wild boar and 25.4% for fallow deer. Nevertheless, still there was no statistically significant difference among species ($X^2_{4,138}$=9.42, P=0.051). In an even closer view at the inland municipality of Gnesta, where 53 of the 143 samples from Södermanland originate and all the species were represented, the difference in the TBEv antibodies prevalence among the species is still no significant ($X^2_{4,51}$=6.92, P=0.14).

Age and gender affecting antibody presence in the species

Answering the question about whether gender and age play a role in the vulnerability of infection of the individuals, the results depend on the species observed. As I explained in the material and methods, I used model selection to check these factors.

For four of the species, more than one model is equiprobable (ΔAICc < 2), whereas for red deer the Age model is the best (See table 2 for the model built and table 4 for the models of the five species). The variable age is included in models selected for all the species except roe deer, and its estimates are positive for all the models, that is, there is a positive correlation between the age and the prevalence of TBEv-antibodies in the species (Table 5). Some of the models selected contain also gender as factor, with negative estimates for male moose, roe deer and wild boar, and positives for male fallow deer. These results confirm the pattern shown in the figure 1, where the percentage of female infected is higher than in males for moose, roe deer and wild boar, and vice versa for fallow deer.

Table 3. Number of individuals positive for TBE for each species and in nine municipalities. Total of individuals collected in brackets; Empty cells means that no samples were collected. Between brackets the initials of counties: D, Södermanland; H, Kalmar; AB, Stockholm; C, Uppland; AC, Vasterbotten.

Municipality	**Species**				
	Fallow deer	Moose	Red deer	Roe deer	Wild boar
Gnesta (D)	5 (10)	6 (8)	7 (16)	4 (6)	4 (16)
Hylte (H)					1 (14)
Mariefred (D)	1 (2)		1 (1)	1 (1)	8 (24)
Nyköping (D)	9 (47)	0 (1)	2 (6)	0 (1)	3 (4)
Haninge (AB)		3 (4)		5 (5)	10 (11)
Södertälje (AB)					12 (18)
Tierp (C)				1 (1)	
Umeå (AC)		4 (14)			
Östhammar (C)					1 (4)
TOTAL	15 (59)	13 (27)	10 (23)	11 (14)	39 (91)

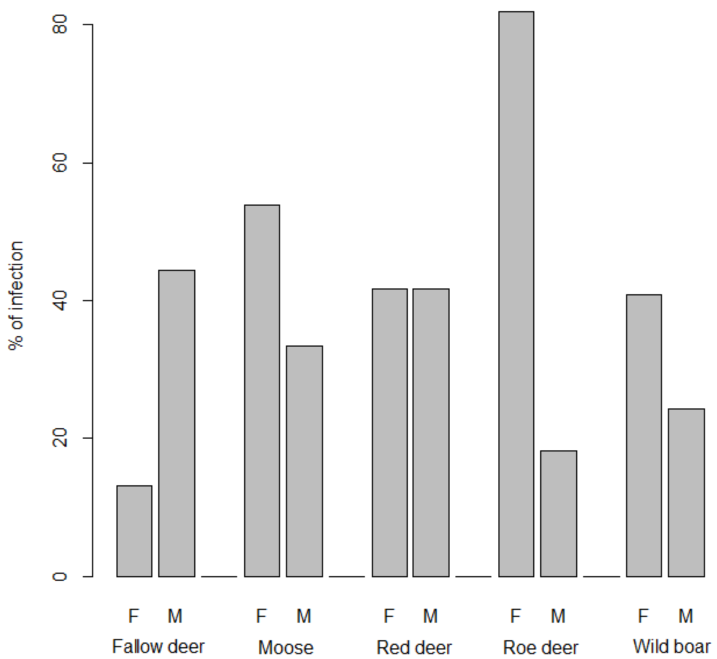

Figure 2. Percentage of infection per sex and species.
F: Female; M: Male.

Table 4. GLMM's built for the model selection based on AICc. Highlighted models have the lowest AICc and ΔAICc lower than 2.

Model	AICc	ΔAICc	wAICc
Fallow deer			
Full	59.6	2.4	0.15
Gender,Age	57.2	0.0	0.51
Gender	61.0	3.8	0.07
Age	58.6	1.4	0.25
Null	65.3	8.1	0.01
Moose			
Full	47.6	2.1	0.10
Gender,Age	46.8	1.2	0.16
Gender	45.7	0.1	0.28
Age	46.8	1.3	0.15
Null	45.5	0.0	0.30
Red deer			
Full	35.5	4.0	0.09
Gender,Age	34.1	2.6	0.18
Gender	39.8	8.4	0.01
Age	31.4	0.0	0.68
Null	37.2	5.7	0.04
Roe deer			
Full	31.9	3.8	0.05
Gender,Age	30.8	2.6	0.10
Gender	28.1	0.0	0.39
Age	29.5	1.3	0.20
Null	29.0	0.9	0.25
Wild boar			
Full	133.9	2.7	0.07
Gender,Age	132.0	0.8	0.19
Gender	131.4	0.2	0.26
Age	132.0	0.8	0.19
Null	131.2	0.0	0.29

Discussion

To the best of our knowledge, this is the first report of prevalence and distribution of tick-borne encephalitis for cervids and wild boar in Sweden. The current literature emphasizes roe deer as the most important large mammal host for *Ixodes ricinus* (Jaenson, 2012a), being a key factor in the distribution of ticks carrying TBEv. In this study, I show for the first time in Sweden, the presence of antibodies against TBE in sera not only of roe deer, but also fallow deer, moose, red deer and wild boar, the main large game species. I found that 34% of the blood samples were antibody positive for TBEv infection, and that prevalence was similar across these species. This clearly indicates that these species are indeed involved in the ecology of TBEv circulation and are an important factor to take in account to understand

Table 5. Statistics of the models with ΔAICc < 2 for every species. In the analysis, 10 from 60 fallow deer samples and 3 from 122 of wild boar were removed due to unknown gender. Also 1 of the 31 moose was removed due to unknown municipality. Significance codes: 0='***' 0.001='**' 0.01='*' 0.05='N.s.'.

FALLOW DEER (2 models) (n=50)						
	Gender and Age			*Age*		
	β	Std. Error	Sign.	β	Std. Error	Sign.
(Intercept)	-2.60606	0.85622	**	-1.98521	0.64254	**
GenderMale	1.55167	0.86923	.			
Age	0.04736	0.02030	*	0.05444	0.01966	**
	Variance of the intercept					
	Variance			Variance		
Municipality	0.454			0.1687		

MOOSE (4 models) (n=30)						
	Gender and Age			Gender		
	β	Std. Error	Sign.	β	Std. Error	Sign.
(Intercept)	-0.36801	1.07107	N.s.	0.2365	0.84908	N.s.
GenderMale	-1.43899	1.03950	N.s.	-1.2678	0.9666	N.s.
Age	0.03460	0.02856	N.s.			
	Variance of the intercept					
	Variance			Variance		
Municipality	1.764			1.41		
	Age			Null		
	β	Std. Error	Sign.	β	Std. Error	Sign.
(Intercept)	-0.91842	0.84908	N.s.	-0.3497	0.6120	N.s.
Age	0.02801	0.02589	N.s.			
	Variance of the intercept					
	Variance			Variance		
Municipality	0.8759			0.6585		

WILD BOAR (4 models) (n=119)						
	Gender and Age			Gender		
	β	Std. Error	Sign.	β	Std. Error	Sign.
(Intercept)	-2.23992	1.02797	*	-1.8211	0.9608	.
GenderMale	-0.73071	0.50677	N.s.	-0.6886	0.5002	N.s.
Age	0.02070	0.01713	N.s.			
	Variance of the intercept					
	Variance			Variance		
Municipality	3.907			3.965		
	Age			Null		
	β	Std. Error	Sign.	β	Std. Error	Sign.
(Intercept)	-2.58214	1.02232	*	-2.2006	0.9651	*
Age	0.01902	0.01690	N.s.			
	Variance of the intercept					
	Variance			Variance		
Municipality	4.08			4.229		

Table 5. Cont.

ROE DEER (3 models) (n=22)						
	Gender			Age		
	β	Std. Error	Sign.	β	Std. Error	Sign.
(Intercept)	1.359	1.019	N.s.	-28.8385	29.0468	N.s.
GenderMale	-2.969	1.235	*			
Age				0.3088	0.3681	N.s.
	Variance of the intercept					
	Variance			Variance		
Municipality	0.6195			2299		
	Null					
	β	Std. Error	Sign.			
(Intercept)	-1.654	4.585	N.s.			
	Variance of the intercept					
	Variance					
Municipality	0.454					

RED DEER (1 model) (n=24)			
	Age		
	β	Std. Error	Sign.
(Intercept)	-2.14477	0.92022	*
Age	0.15143	0.07692	*
	Variance of the intercept		
	Variance		
Municipality	0.454		

the distribution and the risk areas of acquiring TBE on both local and global scale. It is certain that these are not the only mammal species involved in the ecology of the virus circulation. Almost any mammal species may be a blood source for *Ixodes ricinus*. As example, mountain hare (*Lepus timidus*) is related with the maintenance of different borrelia species (including *B. burgdorferi s.l.*) in a tick-hare cycle in islands of the Swedish coast where mountain hare is the only mammalian tick host (Jaenson *et al.* 2009). Red fox may also play an important role, not only as host, but also by predating on other host species, i.e. voles, mountain and European hare or roe deer (Lindstrom et al. 1994). However, large mammals are suspected to participate as blood source for ticks and amplifying the tick population, but are not considered to be competent reservoirs. On the other hand, rodents are considered the main host involved in the maintenance of TBEv, mainly *Apodemus* flavicolis and *Apodemus sylvaticus*, but probably also *Myodes glareolus* and *Microtus agrestis*, due to their high abundance and their high levels of tick infection (Labuda et al. 1997, Achazi et al. 2011)

Because population estimates are expensive and difficult to make properly, hunting statistics may be used to observe possible population changes and trends. According to observations in the hunting bag statistics of the recent years, only the Swedish moose population has decreased (in 2012 3500 specimens less were harvested as compared to 2011). Roe deer, red deer and fallow deer populations continue growing constantly, the latter more compared to the others. However, the wild boar population has showed a rapid increase, where 97300 were harvested in 2012, as compared to the 55000 harvested the year before, according to official sources (Jonas Kindberg, personal notification). Knowing the biology and behaviour of wild boar, with few or no predators besides hunting and being a ubiquitous species that it may adapt to many habitats (Rosell *et al.* 2001), this seemingly uncontrolled increase may promote also a further increase in the Swedish tick population. However, the most important effect could be the dispersion of boars and ticks to new areas, carrying the virus to places where it was not previously present. In the hunting season of 2012/2013 the increase of wild boars harvest was extremely high in the counties of Kalmar and Kronoberg (from 4.8 and 6.2 to 16.2 and 16.0 per 1000 ha respectively). Wild boar was also harvested in the County of Värmland in 2011/2012, where none were harvested in previous years. For the rest of the counties in Central and Southern Sweden where wild boar occurs, its presence has escalated to levels never experienced previously (Jonas Kindberg, personal notification)

Both age and gender appear to affect the probability of TBEv infection. If it is assumed that longer life, i.e. longer time of possible exposure to the virus and higher probability to be infected, it seems logical that there were more positive adults than juveniles. An unexpected result is that gender influenced the presence or absence of antibodies, even in places with high TBEv prevalence (more females infected than males in moose, roe deer and wild boar, and vice versa in fallow deer). To our knowledge, there are no studies reporting a gender difference in prevalence for TBEv. Gerth et al (1995) found in Germany a lower TBEv antibody prevalence in female roe deer than in males, but they could not make a conclusion on this observation. A study carried out in Norway by Lillehaug et al. (2003) found differences, although non-significant, in the prevalence of antibodies against herpes-viruses and pestivirues related to gender and age in red deer and roe deer, where the prevalence was higher in males than in females, and in adults than in yearlings and calves. However, these viruses are not tick-borne and therefore are not expected to have a similar ecology. It is probably that the mode of transmission and the questing behaviour of *Ixodes ricinus* are also important. Further studies are needed to discern the processes involved in the prevalence of antibodies related to large blood meals' gender.

Despite the low prevalence of TBEv found in *Ixodes ricinus* (Pettersson et al. 2014), the prevalence in large host mammals is higher in places historically considered as high risk, including. the counties of Stockholm, Uppland and Södermanland (Jaenson et al. 2012b). There are at least two possibilities, not mutually exclusive, to explain this: (i) the Minimum Infection Rate (MIR) calculated for *Ixodes ricinus* underestimate the reality, for instance due to inclusion of uninfected ticks in the endemic foci, poorly developed techniques for detection of virus at low concentration (Pettersson et al. 2014), and/or (ii) substantial tick populations that allow maintenance of the virus in the nature and infect large amount of hosts despite the low MIR values in ticks.

In summary, in our study, fallow deer, moose, red deer and wild boar have similar sero-prevalence for TBEv as roe deer. A geographical location with a low or absent roe deer population may not be free of TBEv, and this is important to consider when a sentinel species is used to estimate risk areas. It is also important to acknowledge that the rapidly growing populations, of e.g. wild boar may further increase dispersion of *Ixodes ricinus* and TBEv to places currently free of them. It could be necessary, in order to control the further spread of the virus and the vector, to consider a change in wild boar management.

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Appendix

Letter with the instruction for the hunters (in Swedish) included in the sampling kit (A filter-paper already soaked with blood is presented as example on the lower right corner)

Tack för att du vill hjälpa till att samla provmaterial för analys av smittämnen i svenskt vilt!

Med hjälp av dessa prover kan vi exempelvis leta efter antikroppar mot smittämnen och göra oss en bild av situationen på olika platser i landet, följa förändringar och visa nya mönster.

Här nedan finns kopior på instruktionen för provtagning och provhantering samt vilka uppgifter som vi vill skall följa med provet tillbaka till oss.

På de filterpapper som skickas i denna vända finns dessutom ett mindre fliterpapper vidhäftat. Vi använder det mindre pappret som "kontroll" eftersom vi sedan länge vet att det fungerar mycket bra. Vi vill också använda det större pappret eftersom det samlar mycket mer blod som vi kan använda till fler undersökningar. Det är alltså viktigt att dränka det lilla fliterpappret i blod och så mycket som möjligt av det större fliterpappret.

Blod skall samlas från nyligen döda djur. Det är viktigt att djuren inte varit frusna, men om det är det enda alternativet så är det bättre än inget blod alls. Om möjligt så samla blod från djuret direkt efter det dödande skottet, eller vad som är dödsorsaken, innan blodet koagulerar/stelnar för mycket. Om nödvändigt så snitta avlivade djuret i hals eller stickhål för att få blod på fliterpappren.

Det medföljer extra blanketter och påsar för torkat filterpapper att använda vid behov.

Kontakta underteckna om/när du behöver fler blanketter etc.

Studien är en del av Sveriges Lantbruksuniversitets Fortlöpande miljöanalys FoMA-Skog.

För frågor ring 0703 76 16 66 oavsett dag eller tidpunkt, eller e-posta gert.olsson@slu.se

Tack för din insats!

Instruktioner för provtagning när du skall samla blod

Provtagningspappret och en kopia av dessa instruktioner och provblankett finns insvetsade i den mer stryktåliga och vattentäta påsen lämplig att ta med under jakt eller eftersök.

1. Bryt förpackningen och ta fram filterpappret ur påsen märkt "Nytt/vått papper"
2. Dränk filterpapper i rent och färskt blod, exempelvis från stickhålet eller brösthåla
3. Lägg tillbaka det blodddränkta pappret i påsen
4. Efter jakt, torka pappret noga i rumstemperatur
5. Fyll i provblanketten så snart som möjligt och undvik förväxling av olika provnummer
6. Placera det torkade blodpappret och provblanketten i den återförslutbara plastpåsen märkt "Torkat papper"
7. Återsänd prov och blankett i det redan frankerade frisvarskuvertet

Tack för din hjälp och medverkan! Om något ovan är otydligt formulerat eller andra frågor dyker upp, så tveka inte att oavsett tid eller dag kontakta Gert Olsson på **telefon 0703 76 16 66 eller gert.olsson@slu.se**

Provinlämnare: ____________________

Telefon: ____________ Provdatum: ____________

E-post: ____________________

Provpapper nummer: ____________________

Viltart: ____________ kön: ____________

Ålder cirka: ____________ Levandevikt cirka: ____________

Ange var provet kommer från, ex. skottplats, så noga som möjligt:

Kommun ____________________

Närmaste ort: ____________________

Ungefärligt avstånd ort - skottplats: ____________

Ungefärlig riktning ort - skottplats: ____________

Om möjligt GPS-position eller annan mer exakt positionsangivelse:

____

Övriga kommentarer:

____

____

Bilden ovan visar ett exempel på perfekt blodfyllt och torkat filterpapper.