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Carmelo Gómez Martínez

Examensarbete i ämnet biologi
Department of Wildlife, Fish, and Environmental studies
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Carmelo Gómez Martínez

Supervisor: Gert Olsson, Dept. of Wildlife, Fish, and Environmental Studies

Examiner: Kjell Leonardsson, Dept. of Wildlife, Fish, and Environmental Studies

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Abstract

Roe deer have been historically considered the main large mammal host for *Ixodes ricinus* ticks and it has been related with the current distribution and prevalence of tick-borne encephalitis virus in Sweden, being the main arguments the roe deer population peak and a series of mild winters in the nineties. Nowadays, roe deer is again at levels similar to the seventies, but the virus incidence and ticks abundance are still increasing. The role 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 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. The results show infection in individuals of the five species, with the highest prevalence in roe deer, followed by moose, red deer, wild boar and fallow deer. However, the differences among the species are not significant. We also found that the age and sex of the individuals affect 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 and they should be taken into account in the management of the wild populations to prevent an increase of the human infections.

Introduction

Last decades, roe deer (*Capreolus capreolus)* has been considered the main responsible of the distribution and abundance of *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), thus, that tick species plays an important role spreading tick-borne pathogenes as 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, so far and to the best of our knowledge, by *Ixodes persulcatus* (Roelandt et al. 2010). The life cycle of *Ixodes ricinus* last 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 due to the ticks usually co-feed forming clusters on specific parts of the host anatomy, and this proximity promote the 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 less easy 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 is not adjusting to the reality, and the MIR is actually higher. In overall, they estimate that one of 360 tick specimens is positive for TBEv in Scandinavia.

This tick species feed on a broad spectrum of domestic and wild species, mainly mammals but also birds and reptiles (Suss, 2003). Focusing on mammals, other species of cervids besides roe deer, predators as red fox (*Vulpes vulpes*) and wild board (*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 like rodents and large hosts like deer, but the adult ticks feed mainly on larger hosts (Medlock et al. 2013), and the role of any of these species may be also important.

A study carried out in Eastern Poland by Cisak et al (2012) suggest that wild boar, instead of roe deer, plays the most important role in the prevalence of TBE in endemic areas. In Sweden, wild boar was part of the 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 97000 wild boars were shot in 2012 (viltdata.se). Therefore, there is a large wild boar population widely distributed at local and regional level in southern and south-center Sweden.

Moose (*Alces alces*) is described as an important tick host in North America, where only one individual can carry more than 50000 winter ticks (*Dermacentor albipictus*) (Franzmann & Schwartz, 2007). A study carried out in Norway where ears from moose, red deer (*Cervus elaphus*) and roe deer were collected and analyzed for ticks conclude that the three species are massively infected with ticks, although the level infestation seems to be lower in moose than the other two deer species (Handeland et al. 2013). In Sweden, moose population started growing significantly from mid-seventies, reaching a peak in 1982, when 183000 moose were shot (Apollonio et al. 2010). The population decreased in the next decade but now, according with the amount of moose harvested in the last years (from 81000 in 2007 to 96000 in 2012) is increasing again and is spread all over the country. (viltdata.se).

Red deer population remained at very low levels in Sweden until the seventies, when it started growing until the actual levels. However, it is still much lower than the population of the other species, with an estimate of approximately 10000 specimens (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 is not widely distributed, could play an important role in the maintenance of tick population in the areas where red deer is prevalent.

Fallow deer (*Dama dama*) population is also increasing substantially since the beginning of the 1990s, before that, the population grew very slowly (Apollonio et al. 2010). However, the population continues relatively low, in comparison to moose and roe deer populations, being a bit more than 31000 the fallow deer shot in 2012 (viltdata.se).

About roe deer, the population achieves the million in Sweden in the nineties mainly due to the sarcoptic mange that affected the red fox and european lynx (*Lynx lynx*) populations, the main predators for roe deer, in the 1970s and 1980s and the mild winters in the early 1990s (Lindstrom et al., 1994; Jaenson, 2012,a). This number started to decrease with the increase of red fox and lynx, and after the harsh winters of 2009/10 and 2010/11, the population has suffered a hard decline (Jaenson et al. 2012a). Thus, in the hunting season of 2012, approximately 96000 roe deer were harvested, similar amount to the moose harvested in the same year (viltdata.se)

This roe deer explosion and the mild winters in the nineties are the main arguments used to explain the *Ixodes ricinus* ticks population growing and expansion further north along the eastern coastal line (Jaenson et al. 2012,a). Today, despite the roe deer population is decreasing to levels similar to the late 1970s (Apollonio et al. 2010), ticks continue being abundant in all their expansion, through 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, the highest incidence ever recorded in Sweden (Jaenson et al. 2012,b), thus the roe deer abundance as most important factor to the distribution and abundance of *Ixodes ricinus* may be not enough to explain the current situation. Furthermore, nowadays wild boar is a “new” blood source spreading quickly for all central and southern Sweden that it was not present in the seventies, when it was reintroduced to the local fauna, and the other cervid populations have grown significantly in the last decades (viltdata.se).

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 shed further light on the current role of these five large mammal species on the distribution of tick-borne encephalitis virus and prevalence of infection within each species. The following questions are addressed:

1. Are the species equally to TBEv infection? i.e. are there any differences on the levels of infection between the species studied?
2. Does gender and/or age influence on the probability of TBEv infection?

Material and methods

Study area

Our study was performed at several places in southern Sweden (from 60ºN to the south coast, except Holmön Island, at 63N43’). The southern region is historically the most affected by TBE in Sweden, and by including the northern island of Holmön also stretching further north to better overlap the *Ixodes ricinus* tick distribution. Nowadays the *Ixodes ricinus* range has extended throughout the east coast. However under the 60N it is still where more cases of infection are registered, being the Stockholm, Södermanland and Uppland counties the most affected (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. 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 different climate, with a significantly 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 obtained through the years 2010 to 2013 by hunters in several municipalities of 10 counties (Table 1), during the respective 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 while gutting the respectively animals shot, 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 with instructions, and with no further biohazards involved (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 company. 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 on the levels of infection among the different species, we used R (R Development Core Team, Austria, 2008) to build a general linear model with species as independent variable and the presence or absence of antibodies as response variable, and executing an ANOVA test after that. We do this at different scale levels, narrowing the analysis from the "whole" country to a local view. We also test gender and age as factors having an influence on the probability of infection in each species. To do that, we compare five general linear mixed models including all 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. We correct for this "risk" adding the variable municipality as fixed factor. Despite the municipalities are just administrative regions with no dispersal borders of biological significance, and the animals can cross these imaginary lines, we use municipality just as geographical tool to locate the samples collected. We calculated the corrected Akaike´s information criteria (AICc), ∆AICc and AICc in order to estimate which of the models fit better to the data. We considered that models with ∆AICc lower than 2 are equally valid. (Bolker et al. 2009)

Table 2. List of the models built for each species. The names are only to use as 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 antibody presence in 9 of the 31 municipalities where samples were collected (Table 3). All the places are located in south and south-central Sweden except one in northern Sweden. Specimens of five species were found positive, with a higher 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 a general view, there were no significant differences in the level of infection between species ($X^2_{4,254}$ = 6.33, P = 0.17), varying between 30-50%, and the grand total TBEv infection is 34%. In order to have a narrow view, we analysed the samples from the Södermanland County where the 55% of all the 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 if gender and age play a role in the vulnerability of infection of the individuals, the results depend on the species observed. As we explained in the material and methods chapter, we made a 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 the different species and in the nine municipalities found them. Total of individuals collected in brackets; Empty cells means that no blood samples of the species 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)

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

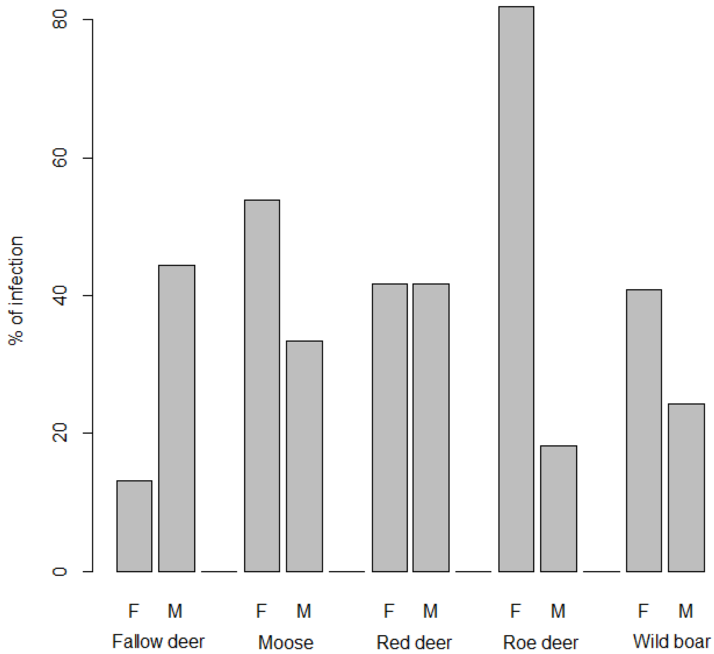

Figure 1. Percentage of infection per sex and species.
F: Female; M: Male.

Table 5. Statistics of the models with ΔAICc < 2 for every species. From the 60 fallow deer samples, 10 were removed of the analysis due to unknown gender. 1 of the 31 moose was removed due to unknown municipality. Also 3 of the 122 wild boar were removed due to unknown sex. 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		

Table 5. (cont)

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		

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		

Discussion

To the best of our knowledge, no observations on cervids or wild boar regarding the prevalence and distribution of tick-borne encephalitis infections in Sweden has been published. The current literature emphasize 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, we show for 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 in Sweden and measure the levels of infection for every species. We found that 34% of the blood samples were antibody positive for TBEv infection, and that antibodies were found in the five species at similar levels. This clearly indicate that these game species are indeed all involved in the ecology of TBEv circulation in the wild through ticks, and they are an important factor to take in account to understand 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 possibly 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. Red fox may also play an important role, not only as host, but as well predating on other host species, i.e. voles, mountain and European hare or roe deer (Lindstrom et al. 1994). However, large mammals are suspect to participate just as blood source for ticks and amplifying the tick population, but in general, they are not 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)

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 (viltdata.se). Knowing the biology and behaviour of wild boar, with few or no predators besides hunting, this seemingly uncontrolled increase may promote also a further increase in the Swedish tick population, and probably of higher importance, 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 there was an increase in the number of wild boars harvested per 1000 hectares that it 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 augmented, although lightly, to population levels never experienced in the past (viltdata.se)

Regarding the age and the gender as factors affecting the probability of TBEv infection in the individuals, both seem to play a role. 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 obvious that there were more adults than young ones observed positive to TBEv infection. An uncommon result is that gender is influencing the presence or absence antibodies, even in places with high TBEv prevalence. To our knowledge, there are no studies explaining different antibody prevalence depending on the gender. 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 types of virus are not tick-borne, and if the differences between genders are due to ecological or behavioural questions, it is probably that the way of transmission and the questing behaviour, i.e. “wait and ambush”, 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 high at places historically considered as “high risk areas”, e.g. 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* does not adjust to the reality, for instance due to inclusion of uninfected ticks in the endemic foci, not well developed techniques to detect virus present at low concentration (Pettersson et al. 2014), and/or (ii) substantial tick population 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 present similar levels of prevalence to TBEv antibodies as do roe deer. A geographical location with a low or absence roe deer population may not be free of TBEv, and this is important to be taken in account when a sentinel species is used to estimate risk areas and other public health related issues. 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. Could be necessary, in order to control the spreading of the virus and the vector, to consider a change in the policies of wild boar management. A better approach on the role the host age and gender in the prevalence of TBEv is also necessary to go further in the knowledge the science has of the tick-borne encephalitis virus.

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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!

<u>Instruktioner för provtagning när du skall samla blod</u>

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 bloddränkta 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: ____________

<u>Ange var provet kommer från, ex. skottplats, så noga som möjligt:</u>

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.

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