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Ecological correlates of a tick-borne disease, *Anaplasma phagocytophilum*, in moose in southern Norway.

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Abstract

As the distribution and abundance of ticks increase, so do the risks of tick-borne diseases. *Anaplasma phagocytophilum*, transmitted by *Ixodes* spp. ticks, is a widespread tick-borne infection causing tick-borne fever (TBF) in domestic ruminants and human granulocytic anaplasmosis. However, the role of wildlife in its epidemiology is poorly understood. Evidence of infection has been detected in wild cervids but the pathogenicity and ecological consequences are unknown. We conducted a serological study of moose (*Alces alces*) in two populations in southern Norway, one where TBF was endemic (Telemark) and the other where sheep ticks (*Ixodes ricinus*) were essentially absent (Hedmark). Seroprevalence to *A. phagocytophilum* antibodies was 79% and 0% respectively. In Telemark, seroprevalence was significantly higher among females that calved successfully (85%) than among others (50%). Body mass and winter mass change were unrelated to serostatus. Relative abundance of questing ticks in Telemark was highest in deciduous forest and lowest in mature coniferous forest, and higher at easterly aspects and altitudes below 350 m. Habitat factors associated with high tick abundance were risk factors for seropositivity among moose. Our findings were consistent with anaplasmosis causing a persistent sub-clinical infection in moose without population-level effects. Further work is needed to establish the importance of moose as a reservoir for the disease in sympatric domestic livestock.

Keywords

Alces alces; climate change; deer; ehrlichiosis; wildlife disease

Introduction

Ixodid ticks, the primary arthropod vectors of zoonotic diseases in Europe, are increasing in abundance and distribution, due partly to climate change (Scharlemann et al. 2008; Jaenson and Lindgren 2011; Jore et al. 2011) and an increasing abundance of wild hosts (Scharlemann et al. 2008; Gilbert 2010). Consequently the risks of tick-borne diseases are also rising (Gray et al. 2009). The rickettsial parasite *Anaplasma phagocytophilum* (formerly known as *Ehrlichia phagocytophila*) is one of the most widespread tick-borne infections in Europe (Stuen 2007). It is transmitted by *Ixodes* spp. ticks with clinical manifestations in domestic ruminants (tick-borne fever; TBF), companion animals and cervids, while in humans it can cause human granulocytic anaplasmosis (HGA), an emerging tick-borne disease (Robinson et al. 2009). Symptoms in cattle and sheep include high fever, loss of appetite, abortion, reduced milk production and, particularly, immunosuppression leading to secondary infections (Alberdi et al. 2000; Stuen 2007; Woldehiwet 2008). As a result, *A. phagocytophilum* affects livestock productivity globally (Lempereur et al. 2011) and it is the tick-borne disease agent causing the greatest economic losses in sheep farming in Norway (Grøva et al. 2011). Anaplasmosis is therefore a disease of socio-economic importance with implications for public health.

The role of wildlife species in the epidemiology of anaplasmosis is not well understood (Robinson et al. 2009), although small mammals and cervids are likely to be important natural reservoirs (Alberdi et al. 2000; Bown et al. 2003). Evidence of infection with *A. phagocytophilum* has been found in many wild ungulates, including moose, across Europe and North America (Magnarelli et al. 1999; Alberdi et al. 2000; Liz et al. 2002; Stuen et al. 2006; Stefanidesova et al. 2008; Robinson et al. 2009). However, little is known of the pathogenesis of *A. phagocytophilum* in wild ungulates (Alberdi et al. 2000), with no clinical signs being observed in wild fallow, red or roe deer in the UK despite antibodies being detected (Stuen 1996). Nonetheless, anaplasmosis was implicated in the death of a moose calf (Jenkins et al. 2001) and the paretic condition of an *A. phagocytophilum* infected roe deer calf (Stuen et al. 2006) in southern Norway. Pathogenicity differs between ungulate species, with a severe clinical reaction observed in experimentally infected reindeer (*Rangifer tarandus*; Stuen 1996) but a persistent subclinical infection observed in red deer (Stuen et al. 2001b). Furthermore, pathogenicity and clinical manifestation may vary with genetic variant of *A. phagocytophilum* (Stuen et al. 2006; Robinson et al. 2009).

Parasite infections in wildlife often have subclinical effects (Gunn and Irvine 2003) but can nevertheless impact host population dynamics if reproductive success is affected (Albon et al. 2002). However, no ecological studies of wild ungulates in relation to *A. phagocytophilum* have been carried out. Our objective was therefore to investigate the relationships between anaplasmosis serostatus and ecological factors, including reproductive success, in 2 populations of moose in southern Norway. One was in the coastal zone (Telemark County), where TBF is endemic in domestic ruminants (Stuen et al. 2006) and moose calving rates have been declining over recent decades (Grøtan et al. 2009). We expected moose here to be exposed to anaplasmosis. The other study area, in Hedmark County, has low sheep tick (*Ixodes ricinus*) abundance (Jore et al. 2011) and we expected moose not to be exposed to anaplasmosis. As symptoms of TBF include loss of appetite and abortion, we were particularly interested in the relationships with body mass and reproductive success, two key factors in ungulate population dynamics (Gaillard et al. 2000). In addition, we described the relative distribution of questing ticks in relation to habitat characteristics and topography. Lastly we related the probability of a moose being seropositive to its use of tick habitat.

Methods

Study areas

Our study areas were located in Siljan and Skien municipalities, Telemark County, southern Norway, (59°21' N, 9°38' E) and in Stor-Elvdal municipality, Hedmark County, south-eastern Norway (61°24' N, 11°7' E; Fig. 1a). Altitude ranged from 20 to 800 m in the Telemark study area and from 250 to 1100 m in the Hedmark study area. In both areas commercially managed coniferous forest, dominated by Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*), was the main vegetation type, interspersed with deciduous stands of birch (*Betula pubescens* Ehrh. and *B. pendula* Roth.), rowan (*Sorbus aucuparia* L.), willow (*Salix* spp.) and aspen (*Populus tremula* L.). Deciduous stands were most abundant in Telemark, but sub-alpine birch woodland occurred above the commercial forest line in both areas. The climate was colder in the more continental Hedmark area. Average monthly January and July temperatures were -2.9 °C and 17.1 °C respectively in Telemark and -8.2 °C and 15.7 °C in Hedmark. Snow cover lasted from December to April in Hedmark and a somewhat shorter period in Telemark.

Both moose populations were partially migratory with current wintering densities of approximately 1.3 individuals km^{-2} (Milner et al. 2012). Red and roe deer occurred in both areas at low densities. At the county level, more than twice as many sheep grazed free-range in summer in Hedmark as in Telemark (Norwegian Agricultural Authority 2012).

Tick sampling

We measured the abundance of questing exophilic ticks in mid-August 2008 in the Telemark study area but not in the Hedmark area where sheep ticks were not encountered during fieldwork or reported by hunters. Based on previous flagging studies in southern

Norway (Jore et al. 2011 suppl. mat.), all questing ticks were assumed to be sheep ticks. Using standard methods (Hillyard 1996), a 1 m^2 piece of white blanket was dragged slowly over the vegetation along 2 parallel 100 m transects spaced 50 m apart. Ticks were counted and removed from the blanket every 10 m. As only nymphs and adults can transmit anaplasmosis (Walker et al. 2001), we excluded larvae from the data presented here. Tick sampling gave an index of active questing ticks, sufficient for relative abundances of ticks between habitats (c.f. Gilbert 2010). Ticks were surveyed in 129 forest stands of differing dominant tree species (pine, spruce or deciduous), age, altitude and aspect, with sampling randomised with respect to time of day.

Moose sampling

Adult female moose accompanied by a calf were immobilised from a helicopter and weighed in a net below the helicopter (Milner et al. 2013). Serum samples were collected from the adults during initial capture and Global Positioning System (GPS) collaring in Telemark in early January 2007 (*n*=18) and 2008 (*n*=15) and in Hedmark in January (*n*=19) and March 2010 (*n*=2). Pregnancy status was determined from serum progesterone levels (Milner et al. 2013). Thirty seven moose were recaptured, reweighed and pregnancy status reassessed in late March of the same year. Spring calving success was monitored by approaching marked females on foot in early June to determine the presence of any new-born calves. Many of the marked moose were harvested between September and December as part of the annual hunting quota, allowing ageing by counting annuli in the cementum of incisor root tips (Rolandsen et al. 2008). Mean age at marking in Telemark was 7.5 years, (range 2.5 - 14.5 years; *n*=25) and 8.5 years in Hedmark (range 3.5 – 15.5 years; *n*=11).

Serum samples were analysed for antibodies to *A. phagocytophilum* by an indirect immunofluorescence antibody assay (IFA) to a horse strain of *A. phagocytophilum* (Stuen et al. 2002) at the Swedish Veterinary Institute, Uppsala. Sera were screened for antibodies at a dilution of 1:40. A titre of 1.6 (log_{10} reciprocal of 1:40) was regarded as positive (Stuen et al. 2002). If positive, the serum was further diluted and retested sequentially to a titre of 1:640. Our expectation was that samples from Hedmark would act as uninfected ‘controls’.

Spatial analysis

Focusing on Telemark, where ticks occurred, collar failure reduced the sample size for spatial analyses to 29 individuals. GPS data were screened for bias (see van Beest et al. 2011 for details, including collar fix success and location accuracy) and individuals were classified as migratory (n=16) or resident (n=13), based on the net displacement distance between the first and subsequent locations (van Beest et al. 2011). Using digital forest stand maps with a resolution of 50 m x 50 m, we determined the forest type at each GPS location used, in terms of dominant tree species and stand age, and recorded the altitude and aspect. We restricted our analyses of habitat use to the period from April to October inclusive, when average monthly mean temperature in Telemark was >5 °C (Norwegian Meteorological Institute data) and ticks were expected to be active (Jaenson and Lindgren 2011). We defined habitat use in terms of the proportion of time each moose spent in each forest type from April to October. We also determined the proportion of time spent at each aspect and in relation to altitude. As serum samples were collected at initial collaring, GPS data were only available for the period after sampling. Therefore, in our interpretation of serostatus in relation to habitat use we made the assumption that an individual’s habitat use patterns were consistent across years.

Statistical analysis

We modelled factors affecting the abundance of questing ticks using a Poisson GLM. As there were many zeros and over-dispersion occurred, we corrected the standard errors using a quasi-GLM model in which the variance was the product of the mean and dispersion parameter (Zuur et al. 2009). Significance was assessed with the *F*-test statistic (Zuur et al. 2009). Potential explanatory terms were altitude, aspect and forest type. Aspect was defined by the four cardinal directions but subsequently some classes were grouped. We initially defined forest type in terms of dominant tree species and stand age but a simplification to four types, namely young deciduous forest (< approximately 60 years), mature deciduous forest (>60 years), young coniferous forest (<40 years) and mature coniferous forest (>40 years), provided the most parsimonious grouping.

To examine the relationship between serostatus and the individual covariates age, body mass at sampling, relative over-winter mass change [log(March mass/ January mass)] and reproductive status in the Telemark population, we grouped individuals into two (seronegative vs. seropositive) and three (seronegative [antibody titre <1:40], seropositive-low [titre 1:40 - 1:160] or seropositive-high [titre ≥1:640]) serological classes. Serostatus was fitted as the explanatory variable in univariate regression models.

We used multiple logistic regression to establish which factors influenced the binomially distributed serostatus (0 seronegative; 1 seropositive) in the Telemark population. Explanatory variables were the significant individual covariates identified above and migration strategy, together with an individual's use of tick habitat in terms of forest type, aspect and altitude. For some variables, low discriminatory power and the limited sample size of seronegative individuals led to non-convergence in multivariate models so their potential importance could only be assessed by univariate models. Furthermore, collinearity between use of mature coniferous forest and use of young coniferous forest was high so we used the one with the greatest explanatory power.

Results

Tick abundance

The abundance of questing nymph and adult ticks sampled in mid-August in the Telemark study area ranged from 0-18.5 per 100 m^2, with a median of 0.5 per 100 m^2. Nymphs were on average 1.3 times more numerous than adults. We found a marked negative effect of altitude such that few ticks were found above 350 m (maximum altitude 619 m), regardless of forest type (Fig. 2, Table 1). Tick abundance was higher in deciduous than coniferous forest, particularly in mature forest, although an interaction with altitude suggested that tick numbers would be highest in young coniferous forests at the lowest altitudes (Fig. 2). East-facing slopes had significantly higher tick numbers than other aspects (Table 1; Fig. 2). Our best fitting model explained 59.3% of the deviance in tick abundance.

Anaplasma prevalence and moose reproductive success

Seroprevalence to *A. phagocytophilum* infection was 78.8% in Telemark (n=33) and 0% in Hedmark (n=21). In Telemark, over half of the females sampled and 73% of those that were seropositive had an antibody titre ≥ 1:640 (Fig. 1).

Within females from Telemark, seroprevalence tended to be higher among pregnant than non-pregnant females (0.85 vs. 0.50; χ^2=3.136, P=0.076; Fig. 1e and Fig. 3) and was significantly higher among females that successfully calved in spring than those that did not (0.93 vs. 0.62; χ^2=4.076, P=0.044; Fig. 1f and Fig. 3). Of 6 seronegative females, 3 were not pregnant and only 1 calved successfully compared with 2 non-pregnant and 11 successfully calving out of 20 seropositive females. We found that pregnant females experiencing prenatal or perinatal losses were no more likely to be seropositive or have a high antibody titre (seropositive-high) than females in other reproductive classes (Fig. 3).

There were no significant differences in either January body mass or relative over-winter mass change between seronegative, seropositive-low and seropositive-high individuals (Fig. 1b-c). Among pregnant females, there was a significant interaction between serostatus and relative over-winter mass change (χ^2=4.873, *P*=0.028). The probability of successful calving was positively related to winter mass change in seropositive females but negatively related to winter mass change in our small sample of seronegative females. While age did not differ between serological classes (Fig. 1d), pregnant females with a high antibody titre were significantly older than other pregnant females (χ^2=4.385, *P*=0.036).

Factors affecting seropositivity

Use of habitats associated with high tick abundance was a risk factor for anaplasmosis seropositivity among moose. Use of mature deciduous forest was positively associated with seropositivity (χ^2=8.969, *P*=0.003) despite overall low usage. Five out of 6 seronegative individuals did not use mature deciduous forest at all and the sixth individual spent 0.02% of its time in this habitat, while median use among seropositive individuals was 0.25%. Fitting problems prevented the inclusion of mature deciduous forest in our multivariate model.

The probability of seropositivity decreased significantly with the proportion of time an individual spent above 350 m altitude (χ^2=3.849, *P*=0.050). This relationship was more pronounced when comparing females with a high titre against other females (χ^2=5.945, *P*=0.015). However there was relatively little individual variation in altitude use, with all but 3 individuals (all of which were seropositive-high) spending over 80% of their time between

April and October above 350 m. Neither mean altitude used nor migration strategy had significant explanatory power.

Reproductive status was a strong predictor of seropositivity and, in our multivariate model, pregnancy status was the term with the greatest explanatory power (χ^2=19.45, *P*<0.001). Pregnant females, and particularly those that calved successfully, had a tendency to use areas below 350 m more than non-pregnant females. We found a significant negative effect of use of mature coniferous forest (χ^2=7.991, *P*=0.005; Fig. 4), the least preferred tick habitat, on seropositivity while the proportion of time spent on east-facing slopes significantly increased the probability (χ^2=4.527, *P*=0.033; Fig. 4). Proportional use of east-facing slopes ranged from 0.05 to 0.72, with medians of 0.14 and 0.28 among seronegative and seropositive females respectively. Together these terms explained 81.9% of the deviance in seropositivity.

Discussion

We found a high seroprevalence to *A. phagocytophilum* infection in moose from our southern study site in Telemark but no exposure in the inland study area in Hedmark where sheep tick abundance is currently low (Jore et al. 2011). A comparably high seroprevalence has previously been reported for moose in Telemark, being higher and with a higher end titre to *A. phagocytophilum* than in moose tested from 3 other counties in Norway (Stuen et al. 2002). The prevalence in Telemark moose was also high compared with other wild cervids elsewhere in Europe (Alberdi et al. 2000; Liz et al. 2002; Robinson et al. 2009; Veronesi et al. 2010; Stefanidesova et al. 2008).

Although TBF in domestic ruminants may cause abortion (Woldehiwet 2008), we found no evidence to suggest that reproductive losses in Telemark moose were associated with the presence of antibodies to *A. phagocytophilum*. Poor autumn recruitment, a feature of our Telemark population, was primarily due to pregnancy failure during mid- to late gestation rather than summer calf mortality (Milner et al. 2013). It therefore seemed unlikely that anaplasmosis was a contributing factor to the declining calving rates observed in Telemark (Grøtan et al. 2009). Early winter body mass and over-winter mass change were unrelated to serostatus. The higher body mass and lower over-winter mass change observed in Hedmark than Telemark reflected large-scale geographic variation in moose body mass across Norway (Herfindal et al. 2006) and poorer winter foraging conditions in Telemark (van Beest et al. 2010; Milner et al. 2013). High titre moose tended to be older than other moose, which was consistent with a higher probability of seropositivity among older sheep (Ogden et al. 2002).

While we have shown that moose in our Telemark study area had a high exposure to anaplasmosis and mounted an immune response to it, a limitation of our study is that we did not know whether animals were clinically infected. Given the high prevalence and tick abundance, it seems likely that moose in Telemark faced repeated tick-borne challenges. In sheep, resistance to experimental re-infection increased with increasing frequency of challenge, but under natural conditions sheep nonetheless showed persistent partial susceptibility to re-infection (Ogden et al. 2002). However, the effects of re-infections are typically less severe than the primary reaction (Stuen et al. 2011). The high *A. phagocytophilum* antibody titre in many of our sampled moose 2-3 months after the end of the tick season, together with the higher seroprevalence among pregnant than non-pregnant females and an absence of effects on body mass, were consistent with persistent subclinical infection, possibly due to re-infection. As conception occurs close to the end of the season of tick activity, the higher seroprevalence among pregnant females was probably not due to a higher probability of infection. Instead suppressed immunity during pregnancy likely meant that pregnant females experienced a more persistent infection or a recurrence of a latent infection. Latency and persistence are features of *A. phagocytophilum* infection due to its ability to infect white blood cells and survive within apparently immune hosts (Woldehiwet 2008). The time seropositive moose take to revert to seronegativity is unknown. In domestic livestock, antibodies to *A. phagocytophilum* generally wane rapidly in cattle, although about a quarter of individuals can remain positive throughout winter (Lempereur et al. 2011). Similarly, TBF can persist from one grazing season to the next in sheep housed indoors over winter (Stuen et al. 2001a).

Despite our small sample size of seronegative individuals, we found strong evidence to suggest that seropositivity was related to the use of preferred tick habitats by moose, if our assumption of consistency in habitat use between years was valid. This has not been well studied although Cederlund & Okarma (1988) stated that adult female moose showed strong fidelity to established home ranges and habitat use was consistent among seasons. Summer habitat use was also highly correlated between years for 3 moose in the Hedmark study area, each with 2 consecutive years of GPS data ($r > 0.97$; B. Zimmermann unpubl. data). As found in Sweden (Lindström and Jaenson 2003; Jaenson and Lindgren 2011), tick abundance was highest in deciduous forest in our study. Consequently use of mature deciduous forest by moose was a risk factor for seropositivity. In addition, we found marked effects of altitude and aspect both on tick distribution and seropositivity which are likely to be related to the microclimatic requirements of ticks (Gray et al. 2009). The relationship between altitude and tick abundance is well known (Gilbert 2010). Evidence suggests that the altitudinal limit has been increasing in recent years in Norway (Jore et al. 2011) and elsewhere (Daniel et al. 2003). In 1983, the altitudinal limit of ticks in the region of our Telemark study was believed to be 150 m above sea-level (Mehl 1983), while we found ticks were abundant up to about

350 m and occasionally found up to 620 m. Assuming that the latitudinal and altitudinal expansion of the tick range observed in Norway (Jore et al. 2011) continues, in parallel with a warmer climate and longer vegetation season (Jaenson and Lindgren 2011), ticks and tick-borne diseases can be expected to move into the Hedmark study area and many other parts of Scandinavia in the near future.

A high abundance of potential hosts is an important factor both for tick expansion (Scharlemann et al. 2008; Gilbert 2010) and, potentially, the spread of anaplasmosis (Alberdi et al. 2000; Rosef et al. 2009). Small mammals can be important reservoirs of anaplasmosis in some systems (Bown et al. 2003) although rodents are thought to play only a minor role in its epidemiology in the parts of Norway where this has been investigated (Rosef et al. 2009). The highest prevalence of *A. phagocytophilum* in questing ticks in Norway occurred in locations with the highest cervid densities (Rosef et al. 2009). As wild cervids have increased dramatically in range and abundance over recent decades and occur sympatrically with free-ranging domestic livestock throughout the summer months in Norway (Mysterud 2000), there is considerable scope for intraspecific disease transmission if, as suspected, cervids are competent reservoirs of *A. phagocytophilum* (Alberdi et al. 2000; Liz et al. 2002; Stefanidesova et al. 2008; Rosef et al. 2009). In our small sample, we found no clear evidence to suggest population-level effects of anaplasmosis on moose in southern Norway. However, the high seroprevalence we found in Telemark warrants further investigation of the competence of moose as a reservoir of infection for domestic livestock and the strains of *A. phagocytophilum* involved. As climate change and the expansion of the tick range continue, there is a need for more detailed research across wild ruminants, and within species over a wider geographical area. This would increase our understanding of the risk factors associated with the transmission of anaplasmosis between wildlife and domestic livestock and improve our ability to manage this widespread tick-borne disease.

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Table 1 Significant factors affecting abundance of *Ixodes ricinus* ticks within the Telemark study area, determined by a Poisson GLM, adjusting for overdispersion with a dispersion parameter of 2.921. Significance was assessed by change in deviance when fitted last, or immediately prior to the interaction term, using the *F*-test statistic

	df	Δ Deviance	*F*	*P*
Atltitude	1	88.04	60.26	<0.001
Forest type	3	57.06	13.02	<0.001
Aspect	1	18.01	12.32	<0.001
Altitude:forest	3	15.48	3.532	0.017
Residual	120	170.4		
Null	128	418.9		

Figure Captions

Fig. 1 Titre to *A. phagocytophilum* antibodies in adult female moose in relation to ecological parameters (mean ± se) in two study areas in southern Norway. Sample sizes are given at the bottom of bars

Fig. 2 Density of ticks (number of adults + nymphs 100 m^{-2}) in relation to altitude (m above sea level) and forest type (young coniferous, mature coniferous, young deciduous or mature deciduous) for a) slopes with an easterly aspect and b) all other aspects within the Telemark study area, August 2008. Points show observed data and lines show predictions from the Poisson GLM given in Table 1

Fig. 3 The proportion of seropositive adult female moose in Telemark in relation to their reproductive status (Not pregnant; pregnant but pre- or peri-natal mortality; successful calving). Sample sizes are given at the bottom of each bar

Fig. 4 The probability of being seropositive to antibodies to *A. phagocytophilum* in relation to pregnancy status and a) the use of mature coniferous forest and b) the use of east-facing slopes in adult female moose in the Telemark study area. Points show observed data and lines show predictions from the best-fitting logistic regression model

Fig. 1

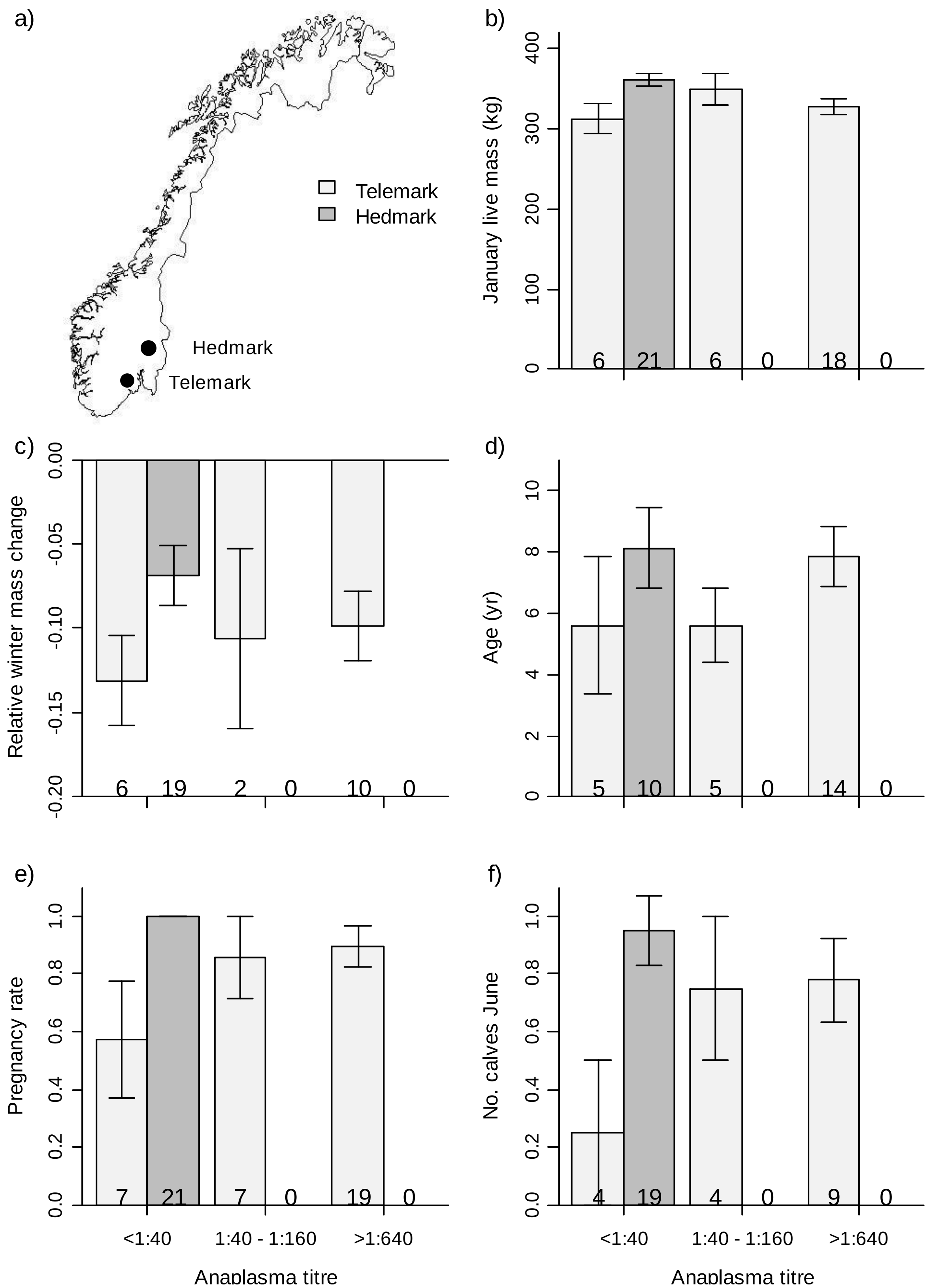

a)
Telemark
Hedmark
Hedmark
Telemark
b)
January live mass (kg)
0
100
200
300
400
6 21 6 0 18 0
c)
Relative winter mass change
0.00
-0.05
-0.10
-0.15
-0.20
6 19 2 0 10 0
d)
Age (yr)
0
2
4
6
8
10
5 10 5 0 14 0
e)
Pregnancy rate
0.0
0.2
0.4
0.6
0.8
1.0
7 21 7 0 19 0
<1:40
1:40 - 1:160
>1:640
Anaplasma titre
f)
No. calves June
0.0
0.2
0.4
0.6
0.8
1.0
4 19 4 0 9 0
<1:40
1:40 - 1:160
>1:640
Anaplasma titre

Fig. 2

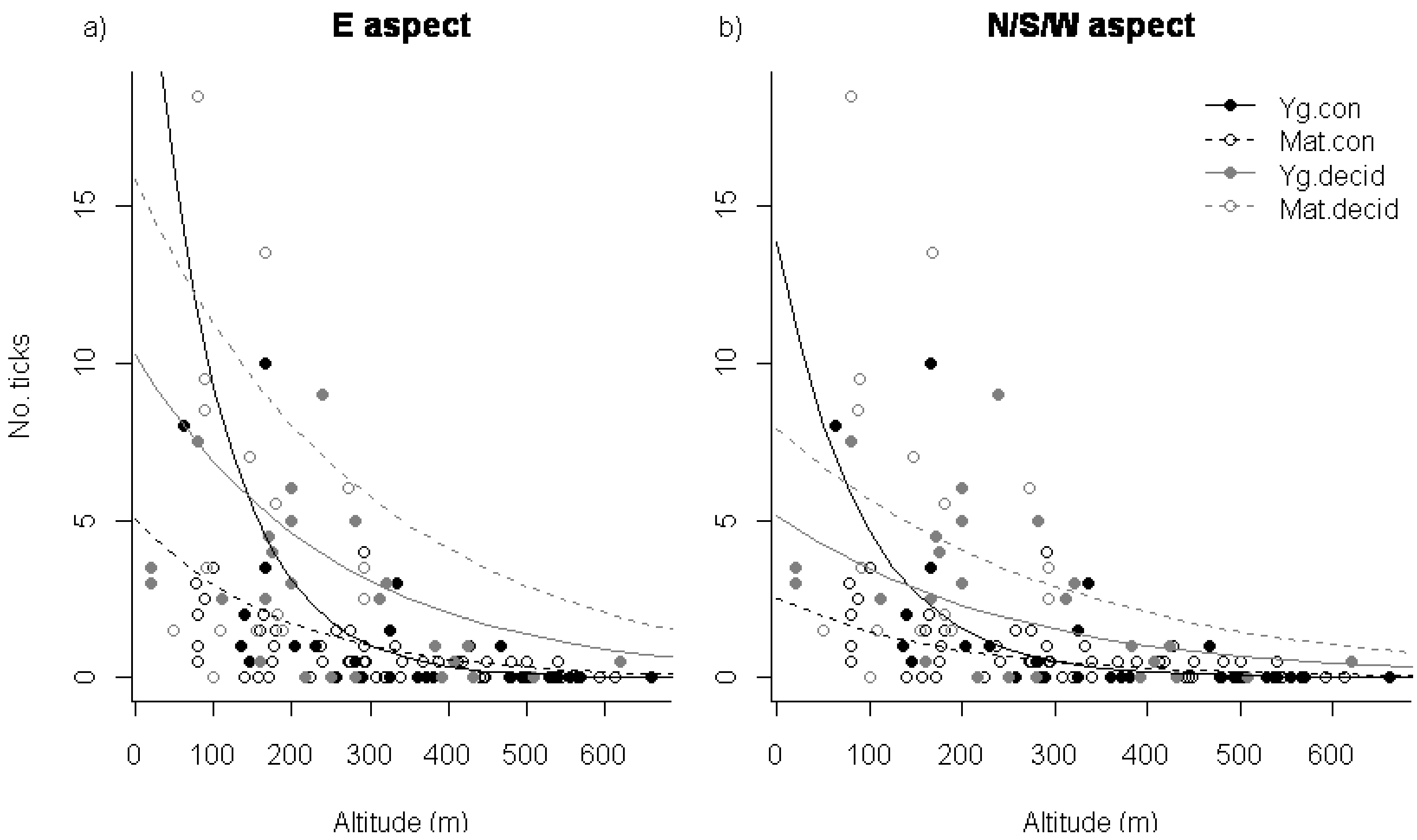

a)
E aspect
b)
N/S/W aspect
Yg.con
Mat.con
Yg.decid
Mat.decid
No. ticks
0
5
10
15
0
100
200
300
400
500
600
Altitude (m)
Altitude (m)

Fig. 3

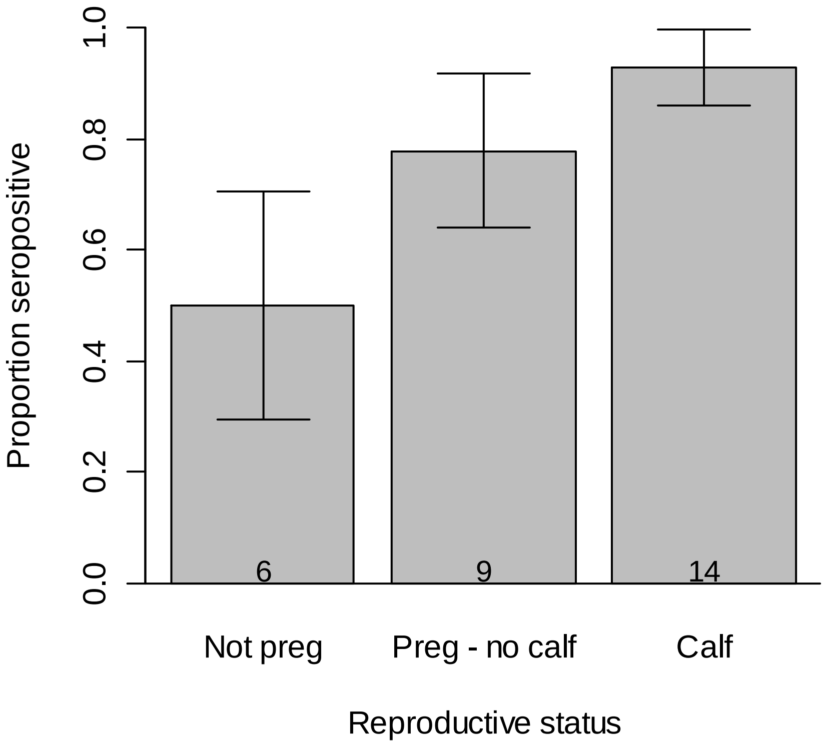
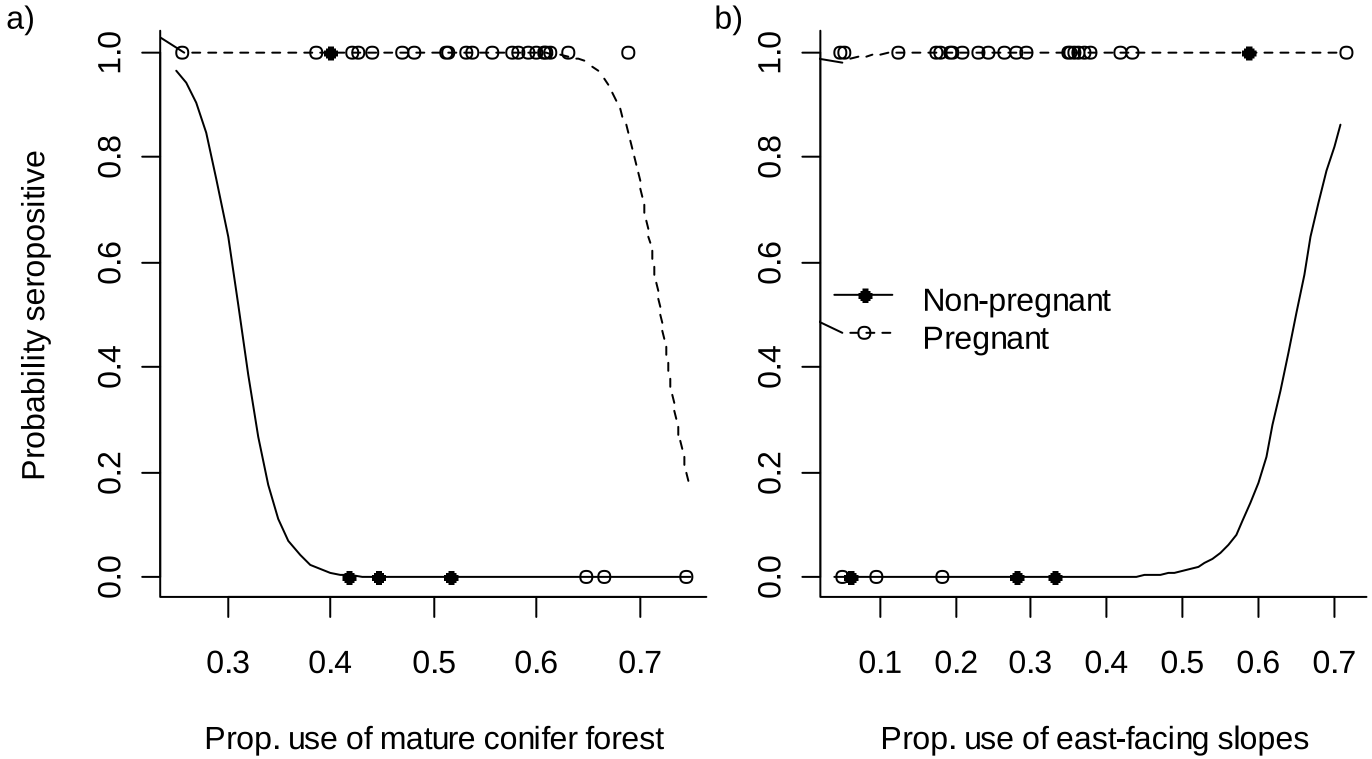

Proportion seropositive
0.0
0.2
0.4
0.6
0.8
1.0
6
9
14
Not preg
Preg - no calf
Calf
Reproductive status

Fig. 4


a)
b)
Probability seropositive
0.0
0.2
0.4
0.6
0.8
1.0
0.3
0.4
0.5
0.6
0.7
Prop. use of mature conifer forest
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Prop. use of east-facing slopes
Non-pregnant
Pregnant