Environmental risk factors for *Ixodes ricinus* ticks and their infestation on lambs in a changing ecosystem: implications for tick control and the impact of woodland encroachment on tick-borne disease in livestock

Lucy Gilbert[1], Kirstyn Brunker[2§], Unni Lande[3], Ingeborg Klingen[4], Lise Grøva[3]

[1] Corresponding author: The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, UK. Lucy.gilbert@hutton.ac.uk

[2]Institute of Biological and Environmental Sciences, Cruickshank Building, University of Aberdeen, Aberdeen, AB24 3UU. UK. Kirstyn.brunker@glasgow.ac.uk

[§]Present address: Institute of Biodiversity, Animal Health & Comparative Medicine, University of Glasgow, Glasgow, G12 8QQ, UK.

[3]Norwegian Institute of Bioeconomy Research (NIBIO), Division of Forest and Forest Resources, 6630 Tingvoll, Norway. Lise.grova@nibio.no unni.lande@nibio.no

[4]Norwegian Institute of Bioeconomy Research (NIBIO), Division of Biotechnology and Plant Health, 1430 Ås, Norway. Ingeborg.klingen@nibio.no

Abstract

Despite global deforestation some regions, such as Europe, are currently experiencing rapid reforestation. Some of this is unintended woodland encroachment onto farmland as a result of reduced livestock pasture management. Our aim was to determine the likely impacts of this on exposure to ticks and tick-borne disease risk for sheep in Norway, a country experiencing ecosystem changes through rapid woodland encroachment as well as increases in abundance and distribution of *Ixodes ricinus* ticks and tick-borne disease incidence. We conducted surveys of *I. ricinus* ticks on ground vegetation using cloth lure transects and counts of ticks biting lambs on spring pastures, where lambs are exposed to infection with *Anaplasma phagocytophilum*, the causative agent of tick-borne fever in livestock. Pastures had higher densities of *I. ricinus* ticks on the ground vegetation and more ticks biting lambs if there was more tree cover in or adjacent to pastures. Importantly, there was a close correlation between questing tick density on pastures and counts of ticks biting lambs on the same pasture, indicating that cloth lure transects are a good proxy of risk to livestock of tick exposure and tick-borne disease. These findings can inform policy on environmental tick control measures such as habitat management, choice of livestock grazing area and off-host application of tick control agents.

Keywords

Woodland expansion, reforestation, bush encroachment, sheep, Anaplasma, tick-borne disease

1. Introduction

Agriculture is the main driver of massive historical and current global deforestation, and this rate of conversion has been accelerating: the net loss of forests increased from 4.1 million hectares per year between 1990 and 2000 to 6.4 million hectares between 2000 and 2005 (FAO and JRC, 2012). However, in some regions of the world such as China and Europe, the opposite trend is occurring: Europe has experienced an increase in forested land cover of more than 600,000 km^2 between 1993 and 2006 (IEEP, 2010). Some of this increase is encouraged through European Union subsidies that incentivise active reforestation in order to improve ecosystem services such as carbon sequestration, water quality and biodiversity. However, much of the expansion of woodland cover in Europe is also unintended. For example, 560,000 Ha (60%) of traditional agricultural Alpine meadows in Switzerland are threatened with natural reforestation or "woodland encroachment" due to a reduction in livestock grazing and hay harvesting (Baur, 2006). The same issue exists in Norway, where reduced agricultural pasture management is allowing the encroachment of woodlands onto agricultural land (Bryn et al., 2012). This is of increasing concern in terms of availability of grazing land for food production, social heritage and tourism (Vinge et al., 2015). Such woodland encroachment onto pastures may also have other consequences, such as the potential to increase the risk of tick-borne diseases to livestock and people, unless tick control measures are implemented (Halos et al., 2010, Gilbert, 2013 and Gilbert, in press).

I. ricinus ticks are generalists, feeding on a wide range of terrestrial vertebrates. Each developmental stage feeds for a few days before dropping off the host to moult and develop or lay eggs in the ground; over 90% of the life cycle of *I. ricinus* is spent off the host (Needham and Teel, 1991). *I. ricinus* are the most important tick species in Europe in terms of vectoring tick-borne pathogens to humans (particularly *Borrelia burgdorferi* sensu lato, the causative agents of Lyme borreliosis, and the tick-borne encephalitis virus) and livestock (particularly Anaplasma, Rikettsia and Babesia species). *I. ricinus* ticks are increasing in abundance and distribution in several areas of Europe (Lindgren et al., 2000; Danielová et al., 2006; Materna et al., 2008), including Norway (Jore et al., 2011). Norway has also experienced an increase in reported incidence of tick-borne diseases such as TBE (Andreassen et al., 2013) and Lyme borreliosis (Hjetland et al., 2014) in humans and tick-borne fever (caused by *Anaplasma phagocytophilum*) which is the most widespread tick-borne infection in animals in Europe (Stuen, 2007). Sheep *Ovis aries* farming is extremely important to livelihoods and economies in rural areas in Norway (SSB, 2013) and one of the main challenges to sheep farmers in the grazing season is the economic loss from tick-borne fever (Stuen and Kjolleberg, 2000; Stuen et al., 2002, 2003; Grøva et al., 2011, 2013), since 55-80% of lambs can be exposed to infection (Stuen, 2001; Grøva et al., 2011) and *A. phagocytophilum*-related mortality rates of over 30% have been observed (Stuen and Kjølleberg, 2000; Brodie et al., 1986). It is thus of increasing urgency to control exposure of livestock to ticks and tick-borne pathogens, and this requires an understanding or where and why tick exposure to livestock is greatest.

Although exposure of livestock to ticks is often mitigated by acaricide (chemical pesticide) application to the host animal (George et al., 2004, 2008; Beugnet and Franc, 2012) there is now an increasing demand for alternative measures to reduce exposure of livestock to ticks such as habitat management, separation of livestock from tick-infested areas or the application of biological control agents to the pastures (Klingen and Duijvendijk, in press). In order to implement these alternative approaches we need to be able to identify which areas or habitats are likely to have the greatest tick abundance and, crucially, whether these areas or habitats are also associated with higher tick infestation on livestock. This latter point is essential to ascertain, because tick abundance in the environment does not necessarily correlate with tick burdens on livestock if the livestock avoid those areas or habitats. If this is the case, applying tick control measures to such areas is unlikely to alleviate exposure of livestock to tick-borne pathogens. However, once we have established which areas are associated with greatest tick burdens on both livestock and in the environment, tick control measures can be implemented in those areas.

Sheep in Norway commonly spend the winter at low altitudes in and around the shelter of the farm buildings. Lambing generally takes place indoors and when the lambs are 1-3 weeks old they are released, together with their mothers, onto fenced spring pastures close to the farm for 2-4 weeks. They are then let out for the summer onto unfenced "rangeland" areas, which generally have a mix of forested and open habitats and are usually at higher altitudes than the farm. Even though the sheep spend only 2-4 weeks on lowland fenced spring pastures, infection rates of lambs with *A. phagocytophilum* (the causative agent of tick-borne fever) during this time is reported to be very high

It is also the enclosed spring pastures where it will be most practical to implement tick control measures; once the sheep are free to roam on the higher altitude unfenced rangelands, controlling where they go or implementing tick control in the environment becomes much more difficult and costly (Klingen and Duijvendijk, in press). Therefore, this study focusses on fenced spring pastures, rather than high altitude unfenced range lands, in Norway.

The overarching objective of this study is to test the effect of woodland encroachment on the risk of exposure of livestock to *Ixodes ricinus* tick bites, with clear implications for risk of tick-borne diseases and economic consequences for agriculture. We therefore aim to identify the environmental risk factors, with a focus on tree cover, in spring pastures associated with (i) abundance of questing (i.e. host-seeking) *I. ricinus* on ground vegetation and (ii) *I. ricinus* tick burdens biting lambs. Importantly, we also test the relationship between the abundance of questing *I. ricinus* on the ground and tick burdens on lambs; this will also confirm whether surveys of questing ticks on ground vegetation can be used as a useful proxy of tick exposure to sheep for future studies.

2. Materials and methods

2.1 Study sites

Surveys were conducted on fenced spring pastures in Møre and Romsdal county in mid-western Norway (62° 30′ N, 7° 10′ E), an area endemic with tick borne fever. A total of 25 spring pastures for grazing animals (predominantly sheep but also horses or cattle, see Table 1) were surveyed in the second half of May and the first half of June in 2011 and/or 2012; 14 pastures were surveyed in both years. All pastures were visited twice in each year surveyed, 1-3 weeks apart. All pastures were within an altitudinal range of 0-80 metres above sea level.

2.2 Questing tick surveys and environmental parameters

The pastures were surveyed for questing tick abundance using the cloth lure method (Vasallo et al., 2000). This consisted of pulling or “flagging” a 1m x 1m square of white flannel towel attached to a wooden broom handle, and inspecting the cloth for ticks every 2m along a 10m transect. Ticks were counted, removed from the material with fine-tipped forceps, and recorded by life stage (nymph, adult male, adult female). Our pilot studies and previous published work (Qviller et al., 2014; Venancio et al., 2016) have shown that *I. ricinus* was the only tick species found by flagging in Norwegian rangeland and pastures and detailed identification of ticks down to species level was therefore not conducted. Between 20 and 40 drags were performed at each fenced pasture per year (10-20 during each of two visits), and transects were at least 5m apart. The cloth lure method cannot estimate tick population density but instead gives an index of relative abundance of questing ticks useful for comparison between areas (Ruiz-Fons and Gilbert, 2010).

For each 10m x 1m flagging transect we recorded canopy cover using a concave densiometer (Construction Safety Products, LA, USA) to measure the percentage tree canopy cover (Mysterud and Østbye, 1999) to determine whether questing tick abundance is associated with canopy cover at a fine spatial scale. To determine the pattern of questing tick abundance with ground vegetation type, especially grazed pasture grass, at the spatial scale of each flagging transect, we also recorded dominant ground vegetation type. For analysis this was categorised as "grass" or "other". Grass was primarily short grazed grasses, whereas "other" consisted of a wide range of rough vegetation such as tussocks, nettles, ferns (e.g. bracken), shrubs (particularly bearberry *Arctostaphylos uva-ursi* and Vaccinium species), and herbaceous species such as wood sorrel (*Oxalis acetosella*), anemone species and viola species. Flagging was conducted in a semi-random manner to include 6-10 replicates per vegetation type on each pasture per visit, in order to represent each ground vegetation type on each pasture. Ground vegetation height was measured using a sward stick because the cloth lure method may have lower efficiency in higher or denser vegetation, therefore this factor must be accounted for in statistical models of questing tick abundance (Ruiz-Fons and Gilbert, 2010). Three height measurements were taken at the start and end of each 10m x 1m flagging transect and the mean value was used in analysis.

As a proxy for the relative index of abundance of hosts, dung counts of livestock and key wild hosts were conducted in a 1m diameter circular area at the start and end of each flagging transect. For analysis, a host abundance index was estimated as the presence (1) or absence (0) of dung in each dung count plot, averaged over the 10m x 1m flagging transect

(Ruiz-Fons and Gilbert, 2010). These were averaged for each vegetation type in each fenced pasture for statistical analysis.

A further test of the impact of woodland encroachment on questing tick abundance in pastures was to use pasture-level, rather than transect-level, habitat. Each pasture was characterised based on the proportion of the pasture under tree cover, grass cover and "other" ground vegetation. This was first estimated on the ground during the first site visit, then checked for accuracy using aerial photographs, and further checked using land cover data (from the AR5 area resource data set, Nilsen and Bjørkelo, 2012) in a geographic information system (GIS) using ArcGIS version 10.1 (ESRI 2012). The GIS data also provided slope and aspect of each pasture.

Using the land cover data in the GIS we also characterised the proportion of woodland cover in the area immediately surrounding each pasture; for this we used a buffer zone 100m wide encircling each pasture. The rationale for this was that wild tick hosts such as deer and foxes prefer to stay close to cover such as woodlands and so we might expect pastures bordering on woodland to be more frequented by these wild tick hosts.

Tick questing activity is strongly affected by temperature (e.g., Gilbert et al., 2014), therefore to help reduce the impact of this potentially confounded factor, daily measurements of ambient air temperature (°C) were obtained from the nearest weather station (data obtained from eKlima.met.no/) to each pasture and entered as fixed effects; three weather stations were used and these were 8 – 15 km from the pastures. To further reduce the chance of weather conditions on a single day confounding the tick counts each site was visited 2-4 times (twice per spring, with most sites surveyed in two years), and up to three pastures were surveyed on the same day, thus pastures often shared daily weather conditions. Flagging was not performed when the wind was strong (> Beaufort Force 5) nor during persistent rain. When the vegetation was wet the towel used for flagging was changed regularly to a dry one.

2.3 Tick burdens on lambs

Tick counts were carried out on lambs (head, neck, legs, inguinal areas) grazing on 14 of the 25 spring pastures surveyed for questing tick abundance in 2011 and 2012, of which two pastures had ticks counted on lambs in both years (Table 1). None of the sheep were treated with acaricides before being turned out onto the fenced spring pastures. The number of ticks biting lambs were conducted on the same day as were flagging surveys for questing ticks on their pastures, for both site visits (i.e. 2 counts were conducted on each lamb, 1 or 3 weeks apart). The stage of development (adult female or nymphal tick) biting each lamb was recorded.

In order to test which pasture and flock characteristics were associated with tick burdens on lambs, we used the GIS-based pasture characteristics (proportional cover of trees, grass and "other" ground vegetation; slope; and aspect) as well as the number of days the lambs spent on the pasture and the density of lambs on each pasture (lambs per hectare).

2.4 Statistical analysis

Analyses of questing nymphs and adult ticks and nymphs and adult ticks biting lambs were conducted separately using generalised linear mixed models (GLMM) implementing the GLIMMIX procedure in SAS Version 9.4. These tick count data had an overdispersed (zero-inflated) distribution, so a Poisson distribution was specified in the model with the residual of the model entered as a random effect, which adjusts the model to match the residual variance thereby allowing for the overdispersion (Bolker, 2015).

Analysis of questing tick data was conducted at the individual transect level with the number of nymphs or adults counted per 1m x 10m cloth lure transect as the response variable, and each site visit per year specified in the model. In contrast, for models of ticks biting lambs, the response variable was the mean tick burden on lambs on each pasture across the two visits per spring, as a t-test showed that there was no statistical significance between visits ($t=0.107$, $df=10$, $p=0.917$).

All models initially contained the pasture-level environmental variables: slope (steepness of angle of each pasture), aspect (classified as either south (southwest to southeast) or “other”), the proportion of trees (or grass or “other” vegetation cover on each pasture, arcsin square-root transformed and entered separately), the proportion of forest in the 100m buffer zone around each pasture (arcsin square-root transformed), pasture management (grazed by sheep in spring only or grazed by horses or cattle until autumn) and year (2012 or 2013).

The models for questing nymphs and questing adult ticks initially contained the following additional variables collected at the time of conducting the cloth lure transects: vegetation height, vegetation type (dominant ground vegetation on the transect, classified as either grass or “other”), canopy cover over the transect (arcsin square-root transformed), host dung (mean of the two counts on each transect), temperature (mean recorded at the nearest weather station on the day of survey), visit number (either the first or second survey in each pasture for each year). Interaction terms with visit number were initially entered, because questing tick abundance could potentially change in some habitats or areas of the pasture over time as sheep remove ticks from the questing population. Site (pasture name) was entered as a random effect as multiple cloth lure transects were conducted per site.

For models of tick burdens on lambs, additional fixed effects initially entered were: lamb density and area of pasture (entered separately to avoid issues of covariation). Unlike the questing tick data, site (pasture name) was not entered as a random effect because the analysis was at the site level, so there were not multiple records per site.

For all models a backwards stepwise procedure was conducted, whereby each variable was sequentially eliminated from the model if it had $p>0.1$. After this procedure, we conducted a forward stepwise procedure, whereby each previously eliminated variable was sequentially added back into the model. These were rejected if $p>0.1$ or remained in the final model if $p<0.1$. We used this threshold for model inclusion because a variable may still have some influence on the overall model even when it does not itself have a statistically significant (usually taken as $p<0.05$) effect on the response variable.

2.4.1 Correlation between questing ticks and lamb tick burdens

Further analyses were conducted to test for a correlation between lamb tick burdens and questing ticks on pastures. A Poisson distribution was specified with the residual entered as a random effect. This was done at the pasture level, using means of ticks per lamb as the response variable and mean ticks per $10m^2$ cloth lure transect and year as the fixed effects.

3. Results

3.1 Questing tick surveys

3.1.1 Questing nymphs

The total number of nymphs counted over all flagging transects at all sites was 809 in 2011 and 648 in 2012, with an average of 1.1 (± 0.35 SE, range 0-58) nymphs per $10m^2$. There were more questing nymphs if there was more canopy cover, less grass, lower ground vegetation, and fewer dung counted over the 10m x 1m transect. There were also more questing nymphs per transect if the whole pasture was characterised as having more tree cover or less grass cover. There were more questing nymphs with increasing temperature on the day of survey and on the first compared to the second survey visit to each pasture (Table 2). Year, pasture management (i.e. whether grazed by sheep in spring only or by cattle or horses until autumn), slope, aspect and buffer zone habitat were eliminated from the model during the backwards and forwards step-wise procedures due to $p>0.1$. There were no significant interactions with visit number.

3.1.2 Questing adults

We found an equal sex ratio of questing adult *I. ricinus* counted by flagging: in 2011 27 females and 26 males were counted, and in 2012 46 females and 45 males were counted, producing an average of 0.14 (± 0.06 SE, range 0-8) adult (female + male) ticks per $10m^2$. We used adults for analysis. There were more questing adults counted if the transect had more canopy cover, less grass and (weak non-significant trend) shorter ground vegetation. There was also an effect of pasture stock management, with more questing adult ticks counted on pastures where animals were kept throughout the summer (usually horses or cattle) rather than in spring only (always sheep), and more questing adults were counted in 2011 than in 2012 (Table 2). Temperature, dung counts per transect, slope, aspect, habitat cover characterising the pasture and buffer zone habitat were eliminated from the model during the backwards and forwards step-wise procedures due to $p>0.1$. There were no significant interactions with visit number.

3.2 Tick burdens on lambs

3.2.1 Nymphs biting lambs

On average, 0.20 (± 0.05 SE, range 0-17) nymphs were found per lamb. Lambs had higher nymph burdens if their pasture's buffer zone had a higher proportion of forest ($F_{1,13}$=12.51, p=0.0037). While not statistically significant, pasture size remained in the model (a weak trend of higher nymph burdens on lambs from pastures of smaller size; $F_{1,13}$=3.43, p=0.0861). Year, slope, aspect, lamb density, and proportion of trees, grass or "other" vegetation covering the pasture were eliminated from the models due to P>0.1.

3.2.2 Adult female ticks biting lambs

On average, 0.87 (± 0.10 SE, range 0-9) adult female ticks were found per lamb (adult males were not counted as they tend not to feed on hosts but instead find hosts to mate with females; they can therefore be difficult to determine in the field while checking lambs). The vegetation cover characterising each pasture had a significant association with adult tick burden (Fig. 1): lambs had higher adult tick burdens if their pasture had a higher proportion of tree cover ($F_{1,13}$=21.64, p=0.0005) and (in a separate analysis) less grass cover ($F_{1,13}$=16.02, p=0.0015). There was no effect of "other" vegetation cover on lamb tick burdens ($F_{1,13}$=0.28, p=0.6058). Year, slope, aspect, lamb density, pasture area and buffer zone habitat were eliminated from the models due to P>0.1.

3.3 Correlation between questing ticks and lamb tick burdens

There was a highly significant positive association between mean adult tick burdens per lamb and mean counts of both questing adult (female + male) ticks ($F_{1,14}$=16.98, p=0.0010) and questing nymphs ($F_{1,14}$=14.01, p=0.0022) from cloth lure transects on each pasture (Fig. 2). Mean nymph counts on lambs significantly correlated with the mean counts of questing nymphs ($F_{1,14}$=11.70, p=0.0041; Fig. 2) but not with those of questing adult (females + males) ticks ($F_{1,14}$=0.96, p=0.3445) from cloth lure transects on each pasture.

4. Discussion

The main aim of this study was to assess the potential impact of woodland encroachment into pastures on tick bite risk (with implications for tick-borne diseases) for sheep in Norway, by examining the associations between tree cover on spring pastures on both questing ticks and lamb tick burdens.

We found that both questing nymph and questing adult tick abundances were positively associated with the proportion of tree canopy covering the 10m x 1m cloth lure transects and, conversely, negatively associated with the proportion of grass (rather than other ground vegetation types) on transects. Similarly, at the whole pasture level, we found that questing nymph abundance also varied positively with the proportion of a pasture with tree cover and negatively with the proportion of a pasture covered by grass. Thus we found a general positive association between tree cover and questing ticks counted on cloth lure transects. While this study was conducted in western Norway, there is evidence of similar associations of questing *I. ricinus* ticks with tree cover in other heterogeneous landscapes, including southern Norway (Vanwambeke et al., 2016), Scotland (Ruiz-Fons and Gilbert, 2010; Gilbert, 2013, 2016; Gilbert et al., 2012) and France (Halos et al., 2010).

Thus, if ticks are to be controlled in the environment (rather than on hosts) application under tree cover may be most effective. However, if this is to be relevant to exposure of sheep to ticks, it is crucial to confirm that tick burdens on lambs are similarly associated with tree cover. Importantly, we did indeed find that more adult female ticks were biting lambs if the pasture had a higher proportion of tree cover (and therefore less grass cover). Similarly, we found that counts of nymphs biting lambs also correlated with tree cover, being higher if the 100m wide buffer strip surrounding the pasture had a higher proportion of forest. Therefore, all other factors being equal, these results suggest that woodland encroachment into, and surrounding, agricultural land used for livestock grazing may increase both questing tick abundance on the ground and exposure to livestock of tick bites. The strength and exact pattern of the disease risk implications are limited because we did not have information on the prevalences of *A. phagocytophilum* or other tick-borne pathogens in the ticks or in the sheep in this study. However, the prevalence of *A. phagocytophilum* infection in lambs grazing on tick-infested pastures in this area of Norway is high (55-80%: Stuen, 2001; Grøva et al., 2011), suggesting that our findings have clear implications for exposure of sheep to tick-borne fever risk. This is likely to also apply to other tick-borne diseases, since questing tick abundance and/or tick burdens on hosts are frequently documented to correlate closely with disease incidence or pathogen prevalence in hosts (e.g. Laurenson et al., 1997, 2003 for louping ill virus; Hofmeester et al. 2016 for *B. burgdorferi* s.l.). Furthermore, previous research has demonstrated the implications of woodland encroachment for the risk of other tick-borne pathogens, and shown that tree/shrub cover affects pathogen prevalence in the same way as it does questing tick abundance. For example Halos et al. (2010) showed that in France both questing tick density and the infection prevalence of *B. burgdorferi* s.l. (the causative agent of Lyme borreliosis in humans) was higher in *I. ricinus* ticks found in woodlands than on pastures, and on pastures that had more shrub cover . Similarly, in

Scotland, Gilbert (2016) showed that questing tick density, *B. burgdorferi* s.l. prevalence and Lyme borreliosis hazard (the density of infected *I. ricinus* nymphs)) were all considerably higher in woodlands than in adjacent open habitats including pastures. Therefore, although we did not specifically measure pathogens here, our results are likely to be relevant to the risk of several tick-borne diseases in both livestock and humans. Our results are most applicable to regions that have heterogeneous landscapes consisting of mosaics of woodlands and pastures, with *I. ricinus* ticks and endemic tick-borne diseases.

Another key output from our study was the close correlation between questing tick counts on cloth lures and tick burdens on lambs, further confirming that any potential environmental tick control measures are relevant to sheep. In addition, it strongly suggests that the commonly used cloth lure tick survey method is a good proxy for biting tick burdens which are reported to correlate with tick-borne pathogen infection rates in hosts (e.g. Laurenson et al., 1997, 2003; Hofmeester et al. 2016).

Additional factors, not directly related to reforestation, also correlated with questing *I. ricinus* abundance on pastures. More questing nymphs and adults were counted on transects that had lower ground vegetation. This is likely to reflect the adverse effect of higher ground vegetation on the effectiveness of flagging as a tick survey method, since *I. ricinus* do not always quest on top of the vegetation so do not always make contact with the cloth lure(Ruiz-Fons & Gilbert, 2010).

Higher temperatures on the day of survey had a positive effect on the number of questing nymphs counted. This should be expected, since low temperatures inhibit tick activity (e.g. MacLeod, 1935; Clark, 1995; Tagliapietra et al., 2011) and, for the temperature range experienced during this study (5-17°C), increasing temperatures strongly increase the proportion of *I. ricinus* ticks that are active (Gilbert et al., 2014; Tomkins et al., 2014).

More questing nymphs were counted on transects where fewer dung piles were recorded and more questing nymphs were counted on the first visit compared with the second visit each year. Dung counts relate to the usage of an area by the animals, while visit number is also related to the amount of time the animals have been using the pasture (livestock had been on the pastures for about two weeks longer for the second visit). Therefore, both these effects could result from livestock “mopping up” ticks from the ground, leaving fewer questing ticks to be counted on cloth-lure transects (see Randolph and Steele, 1985 for experimental evidence of this effect in temporary sheep paddocks).

The opposite effect can occur in larger scale or longer term situations, i.e. higher host densities or hosts using an area for a long period of time are often associated with higher tick abundance in the long term because ticks rely on host blood meals to survive and reproduce. Many studies have found positive correlations between questing tick abundance and long-term host abundance or host usage of space, particularly by deer (e.g. Rizzoli et al., 2002; Gilbert, 2010, 2013; Ruiz-Fons and Gilbert, 2010; Gilbert et al., 2012; James et al., 2013; Qviller et al., 2013; Estrada-Peña et al., 2015). Indeed, we found that questing adult tick abundance varied with pasture management, whereby more questing adults were counted in spring on pastures where animals were kept throughout the season until autumn, with fewer questing adults on pastures where sheep were kept for only 1-4 weeks in spring. Ticks on pastures with hosts available all season have much more opportunity to find a host and feed than those on pastures where hosts are available for only 1-4 weeks, so we should expect generally higher tick populations on the “all-season” pastures. This would be reflected by higher counts of questing ticks in spring (as we did indeed find), although we might predict that surveys in autumn might not necessarily find more questing ticks on all-season pastures because many of the ticks would have found hosts and no longer be questing, as explained above (see also Randolph and Steele, 1985).

We found strong effects of woodland encroachment on questing tick abundance and tick burdens on lambs on spring pastures. This has implications for tick-borne disease risk, since 55-80% of lambs in tick infested areas become infected with *A. phagocytophilum* (Stuen, 2001; Grøva et al., 2011). However, our study did not include the higher altitude "range lands" which are often wooded and used for sheep grazing throughout the summer, so future research needs to determine the environmental risk factors associated with the abundance of questing ticks, tick burdens on sheep and pathogen prevalences in these summer range lands. This would enable policy on tick-borne disease risk mitigation to be informed for both types of grazing lands.

5. Conclusions

We found that woodland encroachment into and surrounding lowland livestock pastures in Norway is associated with higher questing tick abundance in the ground vegetation and also, crucially, higher tick counts on lambs. While we did not measure tick-borne pathogens in the sheep or the ticks, there are clear implications for tick-borne disease risk given the high seroprevalence of *A. phagocytophilum* in sheep in many coastal regions of Norway, the general correlation between tick burdens and tick-borne pathogen prevalence in hosts, and the association of tree cover in and around pastures with increased risk of other tick-borne diseases (e.g. Lyme borreliosis). In similar areas with heterogeneous patchworks of pasture and woodland an endemic ticks and tick-borne disease, we suggest that pasture management that maintains larger areas of grass rather than shrubs or trees and preventing woodland encroachment adjacent to pastures could be potential management tools to help mitigate tick-borne disease risk in pastures. This research can also help inform policy on where best to target any potential biological control of ticks in the environment, i.e. our findings suggest that applying agents (such as entomopathogenic fungi) under or near tree cover would be most cost effective.

Acknowledgements

We are indebted to the farmers for allowing access to their pastures and sheep, and to Peggy Haugnes, Anne deBoer, Marius Bless, Thomas Cull and Sean Webber for fieldwork assistance. This study was funded by the Norwegian Foundation for Research Levy on Agricultural Products (FFL) and the Agricultural Agreement Research Funds (JA), the Møre og Romsdal Council and County Governor in Møre og Romsdal through the project TICKLESS (project number 207737). L. Gilbert received addition support from the Scottish Government's Rural and Environment Science and Analytical Services Division (RESAS) and K. Brunker had additional support from the Natural Environment Research Council (NERC) and the University of Aberdeen.

References

Andreassen, A., Jore, S., Cuber, P., Dudman, S., Tengs, T., Isaksen, K., Hygen, H.O., Viljugrein, H., Ånestad, G., Ottesen, P., Vainio, K., 2012. Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. Parasit. Vectors. 5, 177.

Baur, P., 2006. Die Rückkehr des Waldes im südlichen Alpenraum der Schweiz: Hintergründe eines Landschaftswandels. Agrarwirts. Agrarsoziol. 2, 3-26.

Beugnet, F., Franc, M., 2012. Insecticide and acaricide molecules and/or combinations to prevent pet infestation by ectoparasites. Trends Parasitol. 28, 267-279.

Bolker, M.B., 2015. Linear and generalized linear mixed models in Ecological Statistics: Contemporary theory and application, in: Fox, G.A., Negrete-Yankelevich, S., Sosa, V.J. (Eds.), Ecological statistics: contemporary theory and application. Oxford University Press, Oxford, p. 317.

Brodie, T.A., Holmes, P.H., Urquhart, G.M. 1986. Some aspects of tick-borne diseases of British sheep. Vet. Rec. 118, 415-418.

Bryn, A., Hemsing, L.Ø., 2012. Impacts of land use on the vegetation in three rural landscapes of Norway. Int. J. Biodivers. Sci. Ecosyst. Serv. Manage. 8, 360-371.

Clark, D., 1995. Lower temperature limits for activity of several Ixodid ticks (Acari: Ixodidae): Effects of body size and rate of temperature change. J. Med. Entomol. 32, 449-452.

Danielová, V., Rudenko, N., Daniel, M., Holubová, J., Materna, J., Golovchenko, M., Schwarzová, L., 2006. Extension of *Ixodes ricinus* ticks and agents of tick-borne diseases to mountain areas in the Czech Republic. Int. J. Med. Microbiol. 296, 48-53.

E.S.R.I., 2012. ArcGIS Desktop: Release 10.1. Environmental Systems Research Institute, Redlands, CA.

Estrada-Peña, A., de la Fuente, J., Ostfeld, R.S., Cabezas-Cruz, A., 2015. Interactions between ticks and transmitted pathogens evolved to minimise competition through nested and coherent networks. Sci. Rep. 5, 10361.

FAO., JRC., 2012. Global forest land-use change 1990–2005, by Lindquist, E.J., D'Annunzio, R., Gerrand, A., MacDicken, K., Achard, F., Beuchle, R., Brink, A., Eva, H.D., Mayaux, P., San-Miguel-Ayanz, J., Stibig, H-J., FAO Forestry Paper No. 169. Food and Agriculture Organization of the United Nations and European Commission Joint Research Centre. Rome, FAO.

George, J.E., Pound, J.M., Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitol. 129, S353-S366.

George, J.E., Pound, J.M., Davey, R.B., 2008. Acaricides for controlling ticks on cattle and the problem of acaricide resistance, in: Bowman, A.S., Nuttall, P.A. (Eds.), Ticks: Biology, Disease and Control. Cambridge University Press, Cambridge, pp. 408-423.

Gilbert, L., 2010. Altitudinal patterns of tick and host abundance - a potential role for climate change in regulating tick-borne diseases? Oecologia. 162, 217-225. DOI: 10.1007/s00442-009-1430-x

Gilbert, L. 2013. Can restoration of afforested peatland regulate pests and disease? J. Appl. Ecol. 50, 1226-1233. DOI: 10.1111/1365-2664.12141

Gilbert, L., 2016. How landscapes shape Lyme borreliosis risk, in: Braks, M. (Ed.), Ecology and Control of Vector Borne Diseases Volume 5: Ecology of *Borrelia burgdorferi*, and the prevention of Lyme borreliosis. Wageningen Academic Publishers, Wageningen.

Gilbert, L., Maffey, G.L., Ramsay, S.L., Hester, A.J., 2012. The effect of deer management on the abundance of *Ixodes ricinus* in Scotland. Ecol. Appls. 22, 658–667.

Gilbert, L., Aungier, J., Tomkins, J.L., 2014. Climate of origin affects tick (*Ixodes ricinus*) host-seeking behaviour in response to temperature: implications for resilience to climate change? Ecol. Evol. 4, 1186-1198. DOI: 10.1002/ece3.1014

Grøva, L., Olesen, I. Steinshamn, H., Stuen, S., 2011. Prevalence of *Anaplasma phagocytophilum* infection and effect on lamb growth. Acta Vet. Scand. 53, 30. DOI:10.1186/1751-0147-53-30

Grøva L., Olesen I., Steinshamn H., Stuen S., 2013. The effect of lamb age to a natural *Anaplasma phagocytophilum* infection. Small Ruminant Res. 112, 208-215. http://dx.doi.org/10.1016/j.smallrumres.2012.12.009

Guerrero, F.D., Pérez de León, A.A., Rodrigues-Vivas, R.I., Jonsson, N., Miller, R.J., Andreotti, R., 2014. Acaricide research and development, resistance and resistance monitoring, in: Sonenshine, D.E., Roe R.M (Eds.), Biology of Ticks, Volume 1. Oxford University Press, Oxford, pp. 352-381.

Halos, L., Bord, S., Cotte, V., Gasqui, P., Abrial, D., Barnouin, J., Boulouis, H.-J., Vayssier-Taussat, M., Vourc'h, G., 2010. Ecological factors characterizing the prevalence of bacterial tick-borne pathogens in *Ixodes ricinus* ticks in pastures and woodlands. Appl. Environ. Microbiol. 76, 4413-4420.

Hjetland, R., Nilsen, R.M., Grude, N., Ulvestad, E., 2014. Seroprevalence of antibodies to *Borrelia burgdorferi* sensu lato in healthy adults from western Norway: risk factors and methodological aspects. Acta Pathol. Microbiol. Immunol. Scand. 122, 1114-1124.

Hofmeester, T.R., Coipan, E.C., van Wieren, S.E., Prins, H.H.T., Takken, W., Sprong H., 2016. Few vertebrate species dominate the *Borrelia burgdorferi* sl.l. life cycle. Env. Res. Letts. 11, 43001-43016.

IEEP., 2010. Reflecting environmental land use needs into EU policy: preserving and enhancing the environmental benefits of "land services": soil sealing, biodiversity corridors, intensification / marginalisation of land use and permanent grassland. Final report to the European Commission, DG Environment on Contract ENV.B.1/ETU/2008/0030. Institute for European Environmental Policy / Alterra, Wageningen.

James, M.C., Bowman, A.S., Forbes, K.J., Lewis, F., McLeod, J.E., Gilbert, L., 2013. Environmental determinants of *Ixodes ricinus* ticks and the incidence of *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. Parasitol. 140, 237-246. DOI:10.1017/S003118201200145X.

Jore, S., Viljugrein, H., Hofshagen, M., Brun-Hansen, H., Kristoffersen, A.B., Nygård, K., Brun, E., Ottesen, P., Sævik, B.K., Ytrehus, B., 2011. Multi-source analysis reveals latitudinal and altitudinal shifts in range of *Ixodes ricinus* at its northern distribution limit. Parasit. Vectors. 4, 84. DOI:10.1186/1756-3305-4-84

Klingen, I., van Duijvendijk, G., 2016. Biological control of *Ixodes ricinus* by tick pathogens and invertebrates, in: Braks, M. (Ed.), Ecology and Control of Vector Borne Diseases Volume 5: Ecology of *Borrelia burgdorferi*, and the prevention of Lyme borreliosis. Wageningen Academic Publishers, Wageningen.

Koschorreck, J., Koch, C., Rönnefahrt, I., 2002. Environmental risk assessment of veterinary medicinal products in the EU - a regulatory perspective. Toxicol. Lett. 131, 117-124.

Laurenson, M.K., Hudson, P.J., McGuire, K., Thirgood, S.J., Reid, H.W., 1997. Efficacy of acaricidal tags and pour-on as prophylaxis against ticks and louping-ill in red grouse. Med. Vet. Entomol. 11, 389-393.

Laurenson, M.K., Norman, R.A., Gilbert, L., Reid, H.W., Hudson, P.J., 2003. Identifying disease reservoirs in complex systems: mountain hares as reservoirs of ticks and louping-ill virus, pathogens of red grouse. J. Anim. Ecol. 72, 177-185.

Lindgren, E., Tälleklint, L., Polfeldt, T., 2000. Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick *Ixodes ricinus*. Environ. Health Perspect. 108, 119-123.

MacLeod, J., 1935. *Ixodes ricinus* in relation to its physical environment. II. The factors governing survival and activity. Parasitol. 27, 123-144.

Materna, J., Daniel, M., Metelka, L., Harčarik, J., 2008. The vertical distribution, density and the development of the tick *Ixodes ricinus* in mountain areas influenced by climate changes (The Krkonoše Mts., Czech Republic). Int. J. Med. Microbiol. 298, 25–37.

Mysterud, A., Østbye, E., 1999. Cover as a habitat element for temperate ungulates: effects on habitat selection and demography. Wildlife Soc. Bull. 27, 385-394.

Needham, G.R., Teel, P.D., 1991. Off-host physiological ecology of ixodid ticks. Ann. Rev. Entomol. 36, 659-681.

Nilsen, A.B., Bjørkelo, K., 2012. National land cover and resource statistics, in: International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences. XXII ISPRS Congress, Commission I-VIII, 25 August-01 September, Melbourne, Australia. ISPRS Archives Volume XXXIX-B4: 431-435.

Ogden, N.H., Woldehiwet, Z., Hart, C.A., 1998. Granulocytic ehrlichiosis: an emerging or rediscovered tick-borne disease? J. Med. Microbiol. 47, 475-482.

Ostfeld, R.S., Canham, C.D., Oggenfuss, K., Winchcombe, R.J., Keesing, F., 2006. Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. PloS Biol. 4, 1058-1068

Qviller, L., Risnes-Olsen, N., Bærum, K.M., Meisingset, E.L., Loe, L.E., Ytrehus, B., Viljugrein, H., Mysterud, A., 2013. Landscape level tick abundance relative to seasonal migration in red deer. PLoS ONE. 8, e71299. DOI:10.1371/journal.pone.0071299

Qviller, L., Grøva, L., Viljugrein, H., Klingen, I., Mysterud, A., 2014. Temporal pattern of questing tick *Ixodes ricinus* density at differing elevations in the coastal region of western Norway. Parasit. Vectors. 7, 179. http://www.parasitesandvectors.com/content/7/1/179

Randolph, S.E., Steele, G.M., 1985. An experimental evaluation of conventional control measures against the sheep tick, *Ixodes ricinus* (L.) (Acari: Ixodidae). II. The dynamics of the tick-host interaction. Bull. Ent. Res. 75, 501-518.

Rizzoli, A., Merler, S., Furlanello, C., Genchi, C., 2002. Geographic information systems and bootstrap aggregation (bagging) of tree-based classifiers for Lyme disease risk prediction in Trentino, Italian Alps. J. Med. Entomol. 39, 485-492.

Ruiz-Fons, F., Gilbert, L., 2010. The role of deer (*Cervus elaphus* and *Capreolus capreolus*) as vehicles to move ticks Ixodes ricinus between contrasting habitats. Int. J. Parasitol. 40, 1013-1020. DOI:10.1016/j.ijpara.2010.02.006

Schauber, E.M., Edge, W.D., Wolff, J.O., 1997. Insecticide effects on small mammals: Influence of vegetation structure and diet. Ecol. Appl. 7, 143-157.

Stuen, S., 2003. *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) infecton in sheep and wild ruminants in Norway. A study on clinical manifestation, distribution and persistence. PhD thesis, Norwegian School of Veterinary Science, Department of Sheep and Goat Research, Norway.

Stuen, S., 2007. *Anaplasma phagocytophilum* - the most widespread tick-borne infection in animals in Europe. Vet. Res. Commun. Suppl. 1, 79–84.

Stuen, S., Bergstrom, K., 2001. Serological investigation of granulocytic Ehrlichia infection in sheep in Norway. Acta Vet. Scand. 42, 331-338.

Stuen, S., Bergstrom, K., Palmer, E., 2002. Reduced weight gain due to subclinical *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) infection. Exp. Appl. Acarol. 28, 209-215.

Stuen, S., Granquist, E.G., Silaghi, C., 2013. *Anaplasma phagocytophilum* – a widespread multi-host pathogen with highly adaptive strategies. Front. Cell Infect. Mircrobiol. 2, 31.

Stuen, S., Kjølleberg, K., 2000. An investigation of lamb deaths on tick pastures in Norway, in: Kazimìnovà, M., Labuda, M., Nuttall, P.A. (Eds.), Proceedings of the third International Conference on Ticks and Tick-borne pathogens: Into the 21st century. Slovak Academy of Sciences, Bratislava, pp. 111-115.

S.S.B. (Statistics Norway), 2013. Accessible at: www.ssb.no. Last accessed 19th November 2015.

Tagliapietra, V., Rosà, R., Arnoldi, D., Cagnacci, F., Capelli, G., Montarsi, F., Hauffe, H.C., Rizzoli, A., 2011. Saturation deficit and deer density affect questing activity and local abundance of *Ixodes ricinsu* (Acari, Ixodidae) in Italy. Vet. Parasitol. 183, 114-124.

Thullner, F., Willadsen, P., Kemp, D., 2007. Acaricide rotation strategy for managing resistance in the tick *Rhipicephalus* (*Boophilus*) *microplus* (Acarina: Ixodidae): Laboratory experiment with a field strain from Costa Rica. J. Med. Entomol. 44, 817-821.

Tomkins, J.L., Aungier, J., Hazel, W., Gilbert, L., 2014. Towards an evolutionary understanding of host seeking behaviour in the *Borrelia burgdorferi* sensu lato vector *Ixodes ricinus*: data and theory. PloS ONE 9, e110028. DOI:10.1371/journal.pone.0110028

Vanwambeke, S.O., Van doninck, J., Artois, J., Davidson, R. K., Meyfroidt, P., Jore S., 2016. Forest classes and tree cover gradient: tick habitat in encroached areas of southern Norway. Exp. Appl. Acarol. 68, 375-385.

Vassallo, M., Pichon, B., Cabaret, J., Figureau, C., Perez-Eid, C., 2000. Methodology for sampling questing nymphs of *Ixodes ricinus* (Acari: Ixodidae), the principal vector of Lyme disease in Europe. J. Med. Entomol. 37, 335–339.

Venancio, R., Moraes, G.J., Castilho, R.C., Iwanicki, N.S., Moreira, G.F., Grøva, L., Westrum, K., Klingen. I., 2016. Diversity of edaphic gamasine mites (acari: Mesostigmata: gamasina) co-occurring with the tick *Ixodes ricinus* (acari: Ixodida) in pastures of Western Norway. Syst. Appl. Acarol. 21, 385-397.

Vinge, H., Flø, B.E., 2015. Landscapes lost? Tourist understandings of changing Norwegian rural landscapes. Scand. J. Hospital. Tourism 15, 29-47.

Table 1. Site level characteristics for all 25 spring pastures that were surveyed for questing *Ixodes ricinus*. "Grazing management" refers to livestock being kept on the pasture for either 1-4 weeks in the spring only ("spring") or throughout spring and summer before being moved indoors in the autumn ("to autumn").

Site	Questing tick surveys	Livestock type	Grazing management	% trees/ grass/ other	% woodland in buffer	Slope (°)	Aspect	Lamb tick counts
F1	2011, 2012	sheep	spring	53/43/4	19	4	NE	2011
F2	2011, 2012	sheep	spring	41/42/17	25	5	NW	2011
F3	2012	sheep	spring	10/82/8	57	12	NW	2012
I1	2011, 2012	sheep	spring	17/68/15	22	6	S	2011, 2012
I2	2012	sheep	spring	7/86/7	20	14	S	2012
I3	2011	sheep	spring	43/11/46	13	10	S	2011
I4	2011	sheep	spring	3/86/11	28	9	S	2011
MD1	2012	sheep	spring	33/51/16	22	9	SE	2012
MD2	2012	sheep	spring	50/30/20	60	19	S	2012
MD3	2012	sheep	spring	66/25/9	56	17	S	2012
MD4	2012	sheep	spring	31/14/55	64	9	S	2012
MF1	2012	sheep	spring	4/54/42	46	7	NE	2012
MF2	2012	sheep	spring	37/42/21	63	5	N	None
T1	2011,	cattle	to autumn	6/77/16	48	12	S	None

	2012							
T2	2011, 2012	cattle	to autumn	62/35/3	66	15	S	None
T3	2011, 2012	sheep	spring	55/35/10	63	12	NW	None
T4	2011, 2012	sheep	spring	28/53/19	15	9	W	None
TV	2011, 2012	sheep	spring	3/69/28	8	11	SE	2011, 2012
TS	2012	sheep	spring	49/40/11	85	12	S	2012
V1	2011, 2012	cattle	to autumn	67/11/22	8	4	SE	None
V2	2011, 2012	cattle	to autumn	56/30/14	43	10	SE	None
V3	2011, 2012	cattle	to autumn	73/4/23	64	10	SE	None
V4	2011, 2012	horses	to autumn	57/14/29	0	2	E	None
V5	2011, 2012	horses	to autumn	48/42/10	18	4	SE	None
V6	2011, 2012	sheep	to autumn	56/35/9	3	1	SE	None

Table 2 Outputs from generalised linear mixed model of factors associated with the abundance of *Ixodes ricinus* (nymphs and adults) questing on ground vegetation in spring pastures in Norway.

	Estimate	SE	df	F or t	P
Questing nymphs					
Intercept	-2.16	1.14	31	1.89	0.0688
Visit 1	+0.54	0.12	1, 1095	19.17	<0.0001
Veg. height	-0.03	0.01	1, 1093	28.16	<0.0001
Dung	-4.13	1.27	1, 26	10.59	0.0031
Canopy cover	+0.85	0.12	1, 1100	46.60	<0.0001
Veg. type (grass)	-1.23	0.16	1, 1091	59.08	<0.0001
Temperature	+0.17	0.02	1, 1102	59.09	<0.0001
% trees	+3.64	1.18	1, 24	9.44	0.0053
Questing adults (males + females)					
Intercept	-2.92	0.47	40	6.17	<0.0001
Year (2011)	+0.83	0.19	1, 1107	19.42	<0.0001
Management	+2.31	0.65	1, 18	12.49	0.0024
Veg. height	-0.01	0.01	1, 1100	2.96	0.0857
Canopy cover	-0.96	0.19	1, 1106	24.36	<0.0001
Veg. type (grass)	-1.27	0.24	1, 1102	27.25	<0.0001

Veg. height refers to ground vegetation height (cm); dung refers to the mean of the two 1m x 1m dung counts conducted per 1m x 10m cloth lure transect; canopy cover refers to the proportion of canopy overhanging a point on the cloth lure transect (arcsin square root transformed); Veg. type (grass) refers to the dominant vegetation type along each 1m x 10m cloth lure transect; temperature is the mean temperature recorded on the day of survey at the nearest weather station; management refers to the type of animal management of the pasture, either sheep for 2-4 weeks in spring or other animals (cattle or horses) year-round (except winter); %

trees refers to the proportion of each pasture that is covered by trees (arcsin square root transformed). Values for Visit 1 are in comparison with the baseline of Visit 2; for Veg. type (grass) the baseline is Veg. type (other), i.e. all other ground vegetation types not classified as grass; for Year (2011) the baseline is 2012; for Management the baseline is sheep. The intercept has a t-value; all factors have an F-value.

Figures

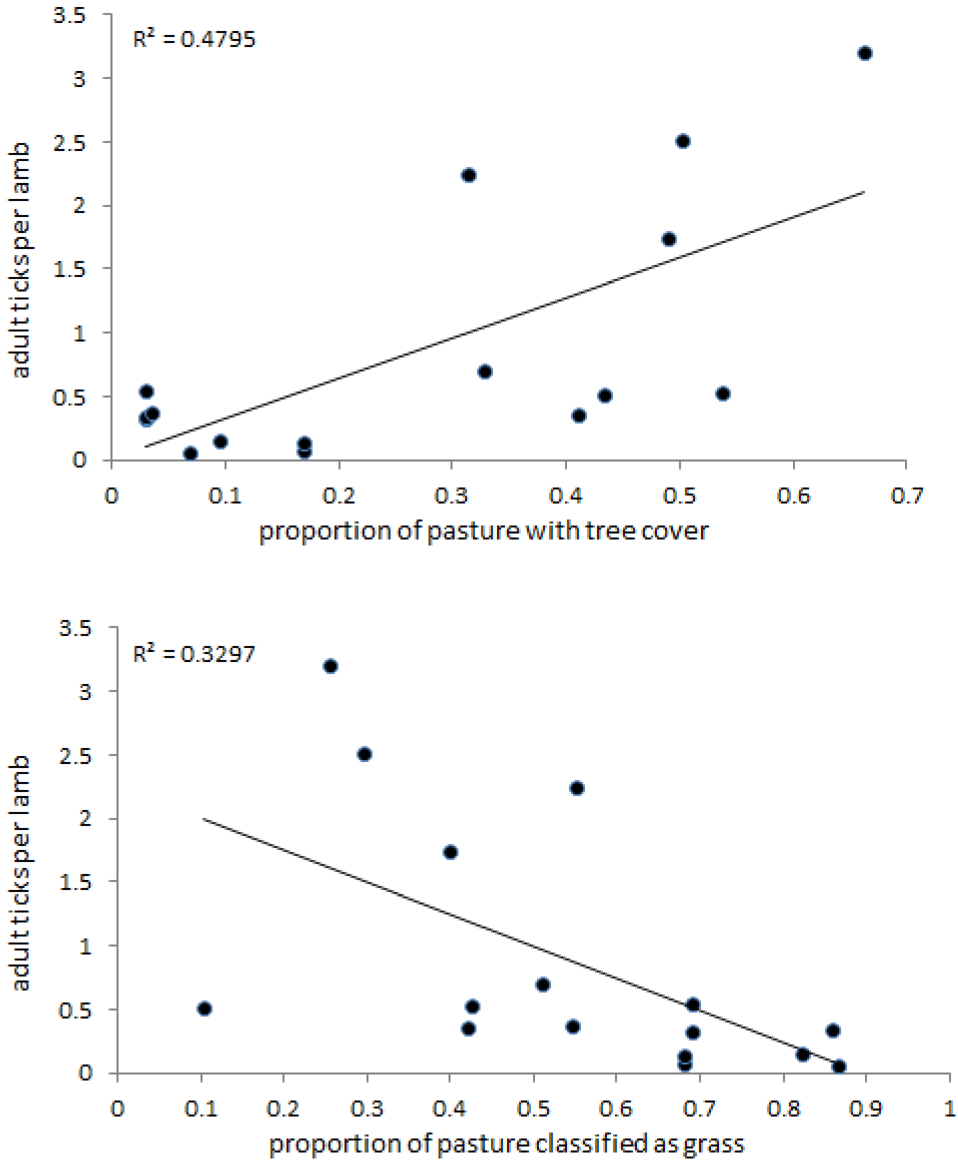

Fig. 1 Relationships between biting adult female *Ixodes ricinus* ticks counted per lamb and the proportion of the pasture covered in (a) trees and (b) grass. Unadjusted raw data are shown.

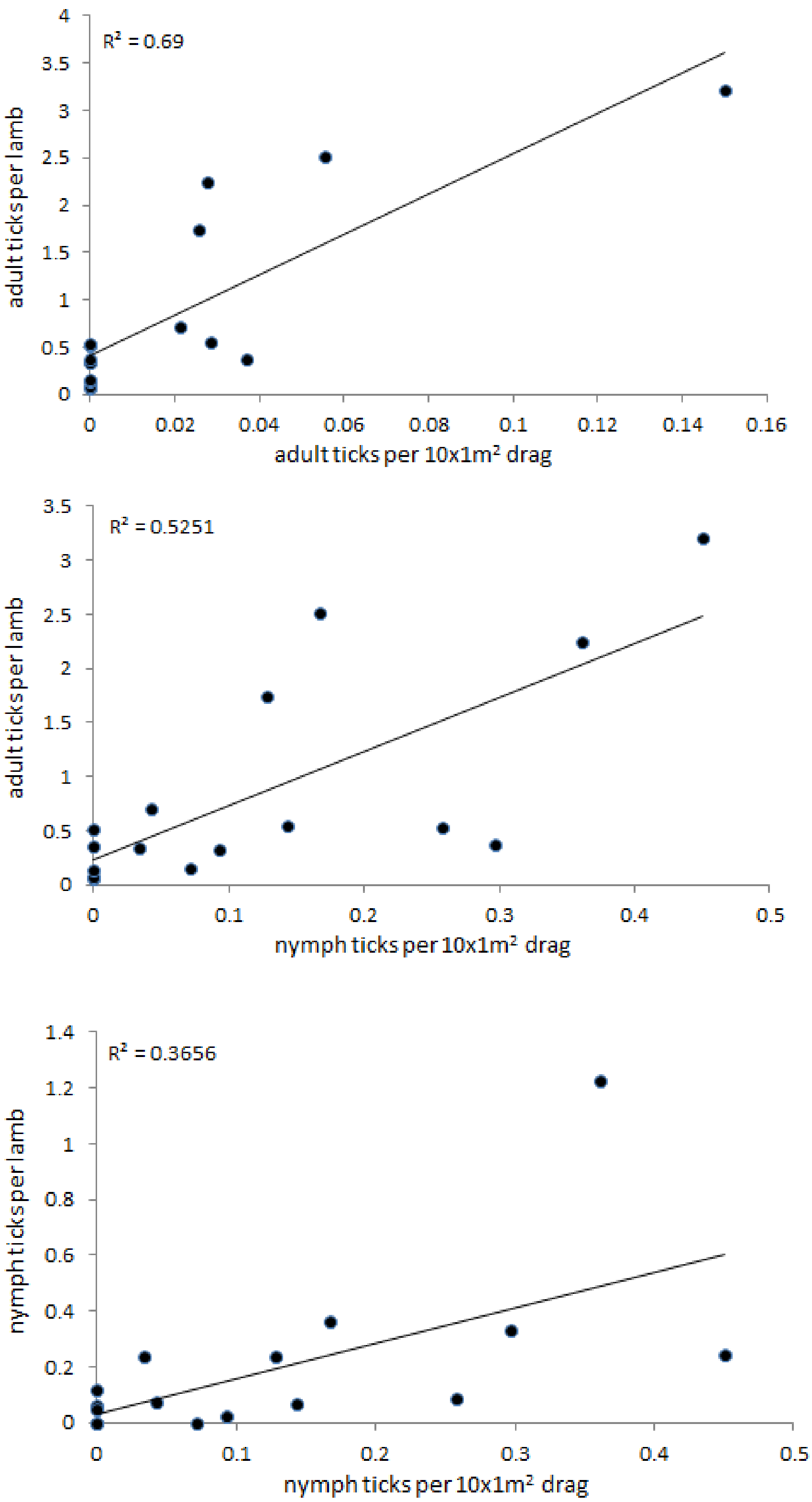

Fig. 2 Relationships between biting *Ixodes ricinus* ticks counted per lamb and questing ticks counted from cloth lure transects for (a) adult females biting lambs and questing adults

(females + males), (b) adult females biting lambs and questing nymphs and (c) nymphs biting lambs and questing nymphs. Unadjusted raw means per pasture are shown.

Figure captions

Fig. 1 Relationships between biting adult female *Ixodes ricinus* ticks counted per lamb and the proportion of the pasture covered in (a) trees and (b) grass. Unadjusted raw data are shown.

Fig. 2 Relationships between biting *Ixodes ricinus* ticks counted per lamb and questing ticks counted from cloth lure transects for (a) adult females biting lambs and questing adults (females + males), (b) adult females biting lambs and questing nymphs and (c) nymphs biting lambs and questing nymphs. Unadjusted raw means per pasture are shown.