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Equine Granulocytic Anaplasmosis in Southern Sweden: Associations with coniferous forest, water bodies and landscape heterogeneity

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ABSTRACT

Landscape characteristics influence both vector and host habitats affecting the spatial and temporal distribution of vector-borne diseases. *Anaplasma phagocytophilum* is one of the most widespread tick-borne diseases in Europe causing tick-borne fever (TBF) in domestic ruminants, and granulocytic anaplasmosis in humans, horses and companion animals. The aim of this study was to identify landscape factors associated with Equine Granulocytic Anaplasmosis (EGA) cases in a refined temporal and spatial analysis by combining land cover data and presence/absence disease data, using a geographical information system (GIS). This study is a retrospective analysis utilizing 1030 EGA diagnostic test results extracted from the National Veterinary Institute (SVA, Sweden). The association between EGA and different land cover types was analyzed with generalized linear models. To analyze the relation between landscape heterogeneity and EGA, we calculated the Gini-Simpson index. Our results showed a significant increase in the proportion positive EGA cases from 2002 to 2015 and marked differences in the seasonal within year distribution of EGA cases. The association with landscape configuration is shown by the positive relationship between *A. phagocytophilum* and coniferous forest, water bodies, and landscape heterogeneity, respectively. The information on the eco-epidemiological drivers for EGA can be central for disease control and prevention. Our method of linking land cover to disease risk may be applied to other vector-borne diseases and to other study regions.

1. Introduction

Diseases transmitted by hematophagous arthropod vectors are a major threat to both human and animal health all over the world (Cerutti et al., 2018). Vector-borne diseases constitute a diverse group of illnesses caused by a virus, bacteria, and other parasites. Their geographical distributions are limited by the distribution of their vectors. However, the presence of a vector is, by far, not the only important factor for the presence of a disease. Landscape characteristics can affect the spatial and temporal distribution of vector-borne diseases, influencing both vector and host habitats (Ostfeld et al., 2005; Lambin et al., 2010). While key elements of the landscape, like different land cover types, affect the habitat suitability for the different species, landscape heterogeneity can affect the spatial and temporal facilitation of vector-host interactions (Hartemink et al., 2015; Real and Biek, 2007).

Microparasite population dynamics is commonly linked to host dispersal, since microparasites typically are less mobile than their hosts (Barrett et al., 2008). The microparasite-host-habitat relationship is especially complex for bridge vectors that have life-cycles involving several hosts. Landscape characteristics shape the mammalian communities available for tick feeding, and the parasite-host relationship influence habitat preferences for both ticks and hosts. In addition, tick-borne diseases create further linkages between epidemiological processes and environmental factors, which can generate complex patterns with large local variation in disease prevalence and risk for infection (Lambin et al., 2010). The prolonged multi-host dependence increases the likelihood for strong relationships between parasitic population dynamics and landscape characteristics (Ogden et al., 2004).

Anaplasma phagocytophilum, an emerging rickettsial parasite, is one of the most widespread tick-borne diseases in Europe causing tick-borne fever (TBF) in domestic ruminants, and granulocytic anaplasmosis in humans, horses and companion animals (Dumbler et al., 2005; Stuen, 2007; Doudier et al., 2010; Woldehiwet, 2010). *A. phagocytophilum* is sustained in nature through enzootic cycles between wild animals and ticks (Stuen et al., 2013). *Ixodes ricinus* (Acari: Ixodidae) is widely distributed in Europe and transmits a large variety of tick-borne
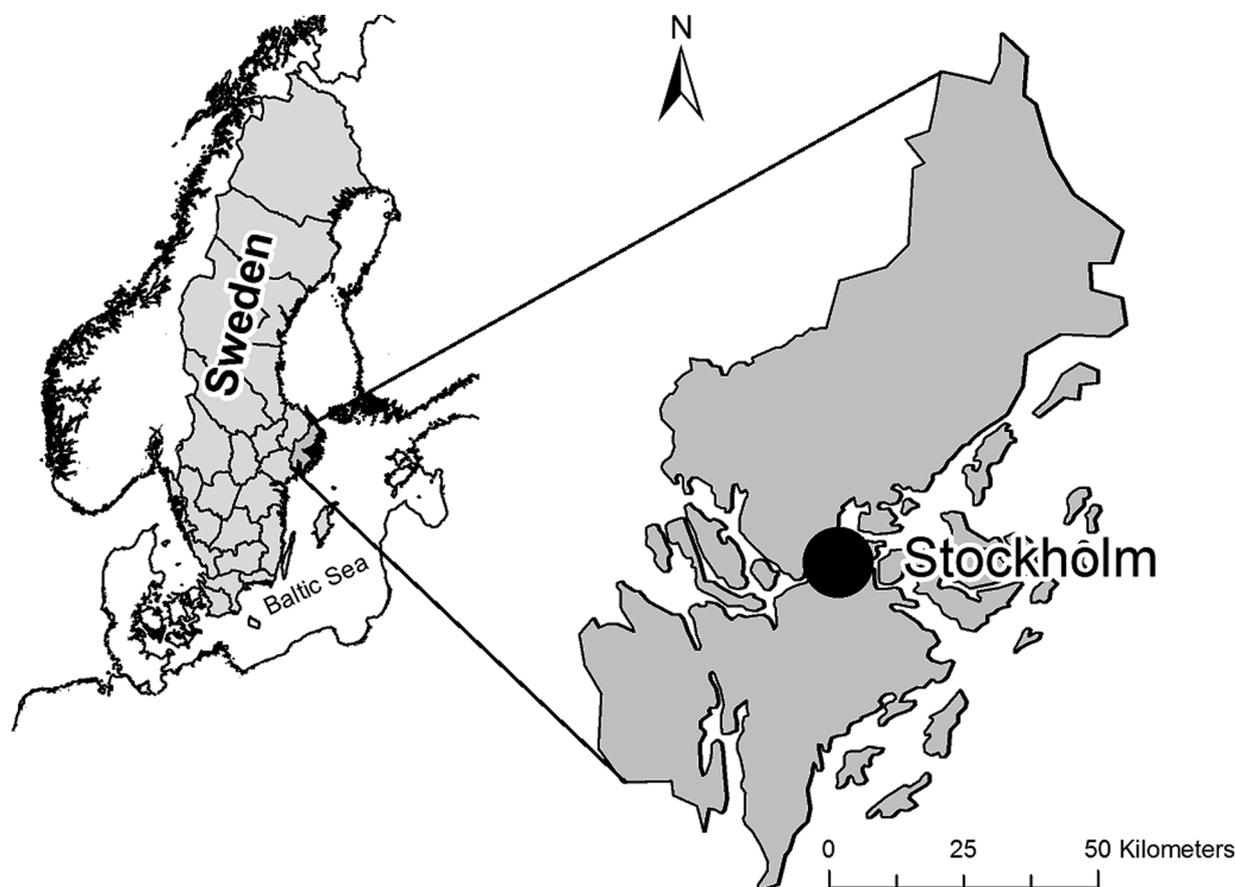
Fig. 1. Map of the study area.

pathogens, including *A. phagocytophilum* (Medlock et al., 2013). *A. phagocytophilum* in *I. ricinus* ticks has been detected in most European countries with prevalence's ranging from 0.4% to 67.0% (Parola et al., 2005; Skarphédinsson et al., 2007). Diseases caused by *A. phagocytophilum* are of large socio-economic importance with severe implications for public and animal health (Milner and van Beest, 2013).

Equine Granulocytic Anaplasmosis (EGA) is a seasonal acute febrile horse disease with an incubation period of 1–2 weeks (Dzięgiel et al., 2013). At the onset, equines may experience clinical signs which include pyrexia, anorexia, depression, limp oedema, reluctance to move, icterus, ataxia, and a decrease in body weight. Mild to severe morbidity, and even mortality, has been detected in some cases of the disease (Franzén et al., 2005, 2009). Recent European studies on the seroprevalence of *A. phagocytophilum* in horses varies from 22.3% in Denmark, 11.3% in France, 8.1% in Italy to 6.5% in Spain. (Leblond et al., 2005; Amusategui et al., 2006; Torina et al., 2007; Hansen et al., 2010; Passamonti et al., 2010). In Sweden, a study from 2001 showed that 16,7% of the horses had antibodies against *A. phagocytophilum* and that seropositivity was strongly correlated with access to pastures (Egenvall et al., 2001).

There can be a great deal of spatial variability in EGA cases since the functional habitat of the pathogen ultimately needs to integrate the functional habitats of vectors and hosts. Woodlands, in particular moist broad-leaved forests, are consistently noted as a key factor in supporting tick and host communities (Ostfedt et al., 2006; Wielinga et al., 2006; Milner and van Beest, 2013; Derdáková et al., 2014). However, *I. ricinus* has also been found in other landscapes such as meadows, pastures and fallow land (Richer and Matuschka, 2011). Small mammals and wild cervids rotate between woodland, meadows, and their ecotones. This movement of animals affects the distribution of ticks and the pathogens they carry (Boyard et al., 2008; Hoch et al., 2010; Ruiz-Fons

and Gilbert, 2010; Li et al., 2014). In addition, land cover changes, such as deforestation, agricultural expansion, and urbanization, may be important indicators of habitat fragmentation defining local scale heterogeneity, which may influence species composition and pathogen presence (Archie et al., 2009; Perez et al., 2016).

Earlier studies have correlated land cover with the distribution of ticks and tick-borne diseases (Brownstein et al., 2005; Milner and van Beest, 2013; Perez et al., 2016; Sándor et al., 2017). However, several of these studies have only used one single level from the CORINE Land Cover classification system (European commission, 2013). CORINE Land Cover classification is a 3-level hierarchical system, ranging from general landscape categories (*level 1*), to more specific land cover classes (*level 3*). In Sweden there is one additional level (*level 4*), with even more detailed land cover classes (Swedish Environmental Protection Agency, 2014). Previous research has suggested that land cover classes at different levels or categorizations may give inconclusive results describing suitable habitats for ticks or tick-borne diseases (Estrada-Peña et al., 2016). Therefore, we propose a hierarchical analysis of the effect of land cover, starting from large land use categories, down to the smallest individual vegetation classes, to analyze habitats that may, directly or indirectly, affect disease transmission of EGA (details provided in Table A1).

Disease transmission may not only be dependent on the classification levels of landscape features, it may also be scale dependent. Different factors affecting disease transmission operate at different spatial scales. Ticks' capacity for horizontal movement is restricted to a few meters during questing (Crooks and Randolph, 2006). Host movement is therefore a crucial factor for the spatial distribution of ticks (Li et al., 2014). Different types of hosts have very different home ranges. Small hosts, like rodents and amphibians, have home ranges limited to a few hundred square meters (Kikkawa, 1964; Kozakiewic et al., 2007),

while larger hosts have home ranges that can extend over several square kilometers (Rivrud et al., 2010). Therefore, an analysis of tick-borne disease distribution needs to involve landscape characteristics measured at different spatial scales.

The aim of this study is to identify spatial risk factors by investigating landscape features related to EGA. In particular, we want to assess at which classification level, and at which spatial scale, different types of habitat affect EGA. We predict that land cover types that integrate the functional habitats of vectors and hosts, as well as landscape heterogeneity per se, can increase the risk of EGA. We also want to assess the temporal variations of EGA in Stockholm County, Sweden during 2002–2015, as well as the yearly seasonal variation in clinical illness.

2. Methods

2.1. Study area

Stockholm County, Sweden (Fig. 1) is situated on the Baltic Sea coastline, comprising cities, suburbs, large rural areas, and an extensive archipelago. The study area is in the hemi-boreal zone, dominated by coniferous forest. Mixed stands with coniferous forest and broad-leaved trees are also rather common. Broad-leaved forest is rare and usually only found close to fields and pastures. Approximately 16% of Stockholm County is agricultural land which includes both fields and pastures. Forest and semi-natural areas cover 48%, and 8% is inland water bodies. In the first half of the 20th century, forests were more open due to grazing by domestic animals. However, decreased grazing in combination with forest management has led to denser forests dominated by conifers (Lindblad and Bradshaw, 1998). Winter temperatures are usually below 0 °C resulting in no, or very low, activity in ticks during December to March.

2.2. Study design

This study is designed as a retrospective analysis utilizing 1030 PCR diagnostic test results for Equine Granulocytic Anaplasmosis (EGA) extracted from the National Veterinary Institute's (SVA) laboratory information system. All clinical samples have been sent to SVA by horse owners or veterinarians, after ocular inspection of horses with symptoms compatible with EGA. SVA is not the only lab performing these tests and there is no compiled information on the total number of tested horses in Stockholm County during the period for this study. Also, there are no regular statistical records of the total number of horses in Sweden neither at national nor at regional county levels. National and regional estimates from the Swedish Board of Agriculture exists for just a few years and the accuracy and precision for these estimates are extremely low (Enhäll, 2017). Due to lack of reliable horse population data we cannot calculate incidence and prevalence for EGA in our study system. Instead we used the proportion of positive PCR results for EGA among all horses that were tested at SVA with symptoms of acute infection, as an estimator of raw incidence.

Serological testing is commonly used to assess a present or past infection by *Anaplasma phagocytophilum*, but it is not useful as a diagnostic test in case of acute clinical symptoms. Therefore, we used PCR to detect an active infection responsible for the symptoms, so as to discriminate from other infectious diseases that can cause the same symptoms. First, we present the proportion of positive test results describing the yearly and monthly variation of EGA positives. Next, we investigate the relationship between landscape features (i.e. different land cover types at different spatial scales, as well as land-cover heterogeneity) and EGA, using a modified case-control approach.

The data were used in two different data frames. All 1030 PCR samples were used to analyse temporal changes during the period 2002–2015. This dataset was also used to investigate if there were any seasonal trends for EGA cases. For spatial analysis, we determined the

proximate case location of as many horses as possible that were clinically tested during 2005 – 2015. In total, we identified 291 geographical positions representing tested horses. For the 291 locations, 92 horses tested positive (EGA cases) and 199 horses tested negative (non-EGA cases). Thirteen of these geographical positions were associated with more than one clinical sample. These different samples either presented different diagnostic results (one location) or the same result but recorded at different points in time (seven locations), or different result at different time points (five locations). Due to the indeterminate nature of these data points we chose to still consider them as independent, and therefore included them in the analysis. The position of each horse was located from Google Earth® and geocoded into latitude-longitude coordinates. Because of confidentiality, there are no maps displaying the geographical distribution of EGA in Stockholm County presented in this study.

2.3. Molecular detection of Anaplasma phagocytophilum

Real-time PCR was used to detect and quantify *A. phagocytophilum* DNA in tick vectors and hosts and is the PCR method used at the Swedish National Veterinary Institute (SVA). From 200 microliters of EDTA blood, DNA was extracted in a BioRobot EZ1 (Qiagen, Hilden, Germany) according to the manufacturer's instructions using the EZ1 Tissue Kit and the bacterial protocol from the same manufacturer. The elution volume was 100 μl and the eluate was used as template in the PCR reactions. Real-time PCR for *A. phagocytophilum* was performed as described by Goodman et al. (1996), with the primers described in the erratum of that paper, but converted to a real-time PCR system by the addition of a Taqman probe (6AMd(CTGTCGTCAGCTCGTGTCGTGAG ATGTTG)BHQ-1). Each 20-μl PCR reaction mixture contained 1× PerfeCTa qPCR FastMix, UNG, Low Rox (Quanta BioSciences Inc., Gaithersburg, MD, USA), 0.4 μM of each primer, 0.15 μM of the TaqMan-probe and 2.5 μl of template DNA. The PCR amplification was performed in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA), with an initial denaturation step at 95 °C for 10 min, followed by 45 cycles of 95 °C for 3 s and 60 °C for 30 s (Goodman et al., 1996).

2.4. Land cover data

To explore the relationship of land cover with the proportion positive results of EGA, pre-existing land cover maps were incorporated within a geographical information system (GIS). Data on land cover for Stockholm County (SMD) were extracted from the Swedish Environmental Protection Agency (Swedish Environmental Protection Agency, 2014). These maps are based on satellite images with a spatial resolution of 25 × 25 m, and the national coordinate system: SWEREF 99 T M. Spatial analysis measurements were made with ArcMap version 10.3 (ESRI, Redlands, CA, USA) to compare the spatial relationship among the different study sites. Although previous research has conducted investigations at different buffer zone sizes, no standard distance for these zones has been determined (Killilea et al., 2008; Li et al., 2012; Jore et al., 2014; Asghar et al., 2016; Gilbert et al., 2017). For each of the 291 study sites we constructed ten successively increasing buffer zones from 100 to 1000 m radii with 100 m increments around each horse location. At all sites we calculated the proportion of each vegetation type within each concentric buffer zone. The smaller buffer zones were chosen to capture the land cover types within the parcel unit of each horse location. The larger buffer zones were created to capture land cover types that reflect the larger context of suitable host habitats within the adjacent land around each farm.

To test the associations between EGA and land cover categories, we compared the proportion positive PCR results with categorical descriptions of the land cover. The same type of research design, using sick test-negatives as controls, has been used to study the association of landscape features with the risk of canine leptospirosis (Raghavan et al.,

2011). For definition of land cover classes, we used the classification of the European landscape from CORINE Land Cover (CLC) 2012 (European commission, 2013). The CORINE classification is based on a hierarchical standard nomenclature of three levels: five broad land cover types at *level 1* (1 – artificial surfaces, 2 – agricultural areas, 3 – forests and semi-natural areas, 4 – wetlands and 5 – water bodies), 15 land cover types at *level 2* and 44 land cover types at *level 3*. In addition, we also used the Swedish land cover classes (*level* 4) represented in the Svenska Marktäckedata (SMD), which has an even more detailed classification of land cover, consisting of 59 land cover and vegetation types (Swedish Environmental Protection Agency, 2014). These four different levels of land cover classifications are compared in Table A1. All classification levels (*Level 1 – 4*) have the same image resolution of 25×25 m pixels. The comparison between levels is thus a matter of classification rather than scale. For each land cover classification level, we derived the proportions of each land cover type from each buffer zone. The proportion of the different land cover types were then used as explanatory variables in generalized linear models with the PCR test result for each horse as response variable.

To be able to study the association between land cover heterogeneity on the proportion positive EGA results, we calculated Gini-Simpson (GS) index (Eq. (1)) for each of the 291 sites using each land cover classification level and each buffer zone size.

$$GS = 1 - \sum_{i=1}^{s} p_i^2 \tag{1}$$

s is the number of different land cover classes and *p* is the proportion of land cover class *i*. We then analysed the relationship with landscape heterogeneity measured as Gini-Simpson index on the PCR results for each horse (n = 291). An example of the effect of the increasing number of classes and its consequences for the spatial configuration in buffer zones is shown in Fig. 2. In the largest buffer zones, with a radius of 1000 m, there can be a high diversity of land cover types. Smaller buffer zones may of course have fewer land cover types, especially when using *level 1*.

2.5. Statistical analyses

To analyze the change in the odds of obtaining a positive EGA sample during the period 2002–2015, we applied a logistic regression with quasi-binominal error distribution to correct for over-dispersion (Crawley, 2007). For the seasonal pattern analysis, we plotted the mean monthly proportion of the annual number of EGA cases for the period 2002–2015 and call this the proportional frequency of cases. In addition, we plotted the monthly mean number of PCR tests per year, and the monthly mean proportion positive EGA results per year.

The association with land cover types and *A. phagocytophilum* PCR results (n = 291) were analysed in 40 separate generalized linear models; for ten different buffer zone sizes combined with 4 different levels of land cover classes. All land cover classes were included in a full starting model in each analysis. Model selection was conducted using the StepAIC function with the BIC criteria for backward elimination of non-informative variables. For easier interpretation of the final models, all non-significant variables were omitted from the result table, even if the variable was retained in the final model. To check for multi-collinearity, we calculated the variance inflation factors (VIFs) for all explanatory variables in the final model (Dobson and Barnett, 2008). It was only at *level 2* in buffer zones with 500 m and 600 m radii that we found VIFs indicating high correlations among some of the land cover types. This was solved by removing the land cover type "Arable land" from these final models. Final model residuals for all levels and explanatory variables were also inspected for presence of autocorrelation based on the Moran's I statistic using the spdep package in R (Bivand, 2011).

The association with landscape heterogeneity measured as Gini-

Simpson index was analysed in the same way as above, with general linear models with the outcome of the *A. phagocytophilum* PCR results (n = 291) as a function of Gini-Simpson index, for each land cover level and buffer zone size, and tested for spatial autocorrelation. All statistical analyzes were performed with R 3.5.0 (R Core Team, 2018), and the additional packages effects (Fox, 2003), car (Fox and Weisberg, 2011), and MASS (Venables and Ripley, 2002).

3. Results

Thirty-two percent of all horses tested for EGA at the National Veterinary Institute (SVA) during 2002–2015 showed a positive PCR result. The proportion positive tests showed a significant increasing trend over the study period (odds ratio = 1.056, p < 0.05, logistic regression; Fig. A1; Table A2).

There were large differences in the seasonal proportional distribution of positive PCR tests for A. *phagocytophilum* for the period 2002–2015. Very few clinical tests were performed during November to April for each year, which could be expected given the seasonal variation in tick activity due to temperature. The mean number of tests was lower than one per month for these winter months. There were a few positive cases in March, April and November, but the bulk of cases at SVA occurred during May to October, with the highest proportional frequency in September (Fig. 3).

3.1. The association of landscape features with EGA cases

Associations with land cover types were evaluated retrospectively based on 92 EGA positive and 199 EGA negative geographical locations in Stockholm County. We did not find any significant spatial autocorrelation of residuals from any of the final models tested in this study. This was true both for the analyses of land cover types and for the analysis of landscape heterogeneity. The relationship between different land cover types and *A. phagocytophilum* varied among the different buffer zone sizes. However, results from the four different land cover levels were congruent (Tables 1 and 2). CORINE Land Cover *level 1* includes five broad land cover categories: artificial surfaces, agricultural land, forest and semi-natural areas, wetlands, and water bodies. Results from the generalized linear models show that several landscape factors have significant positive associations with EGA, which means that the risk for infection is increased. However, there also exist negative associations, indicating a decreased risk for EGA.

At land cover *level 1*, forests and semi-natural areas, and water bodies showed significant positive associations with EGA cases in buffer zones with 400–1000 m radii (Table 1). Agricultural areas had a significant negative association at 200 and 300 m buffer radii (Table 1; Table A3). There were no significant associations between EGA and artificial areas or wetlands (Table 1). It should be noted that at land cover *level 1*, pastures and arable land are grouped together into agricultural areas, and all forests including coniferous forests, mixed forests and broad-leaved forest are grouped together in the category forest and semi-natural areas.

Land cover *level 2* includes 15 land cover classes. Here, we found a significant positive relation between forest cover and EGA, all the way from 200 to 1000 m buffer radii (Table 1). The positive association with inland waters was similar to *level 1*, significant in buffer zones with radii from 400 to 1000 m. At *level 2*, artificial non-agricultural vegetated areas showed a positive association at 100–300 and 600–700 m radii (Table 1; Table A4). At *level 2*, agricultural areas did not have a significant association, and in contrast to *level 1*, at *level 2* this category is subdivided into arable land and pastures.

Land cover *level 3* includes 44 different land cover classes. At *level 3*, the forest category is subdivided into three main categories, broad-leaved forest, coniferous forest, and mixed-leaved forest. At this level, we found that there was still a positive association between forest and EGA at buffer radii 200–300 and 500–1000 m, but only for coniferous
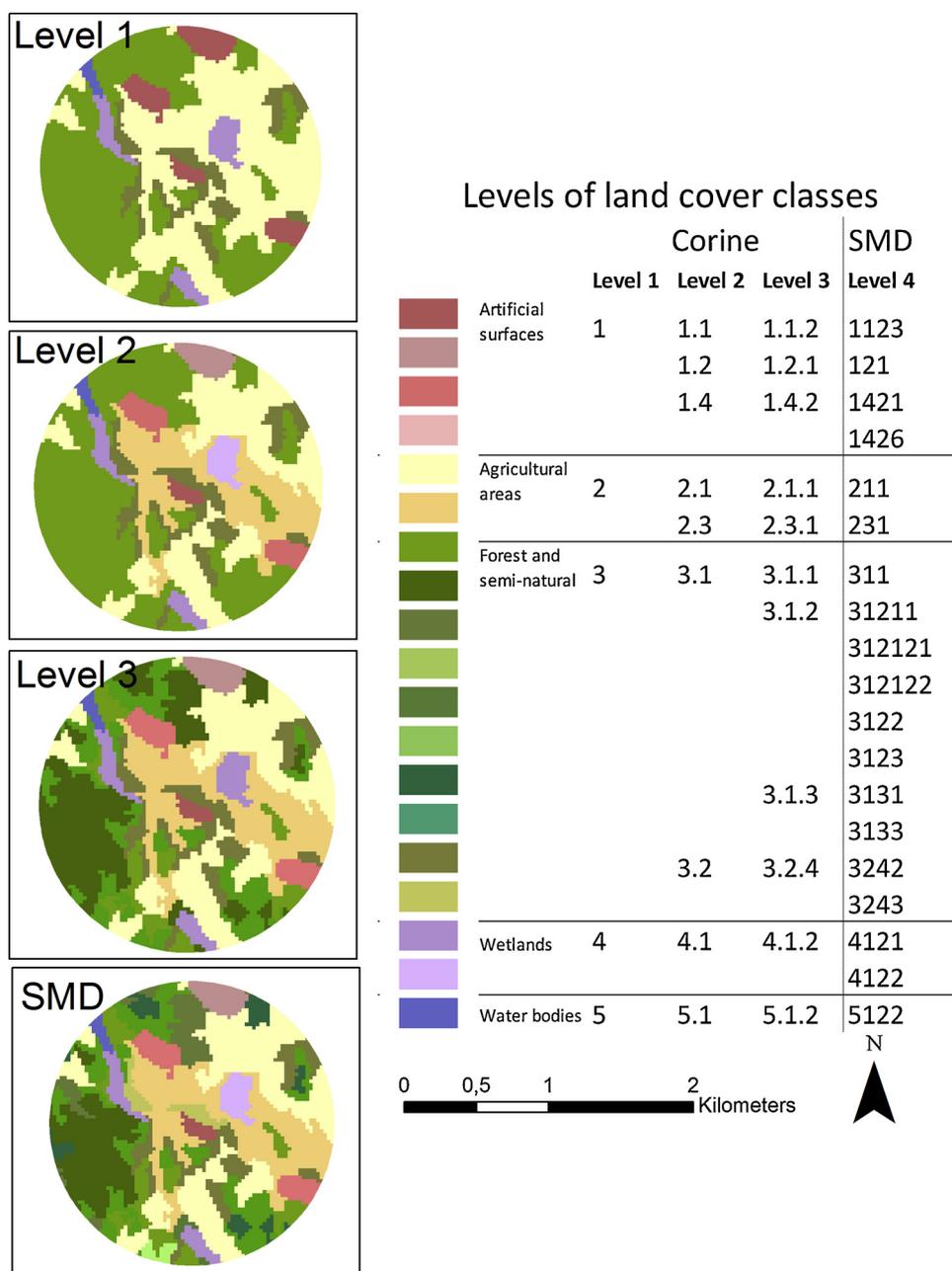
Fig. 2. Example of representation of different land cover types in one buffer zone with a 1000 m radius. The map of land cover types is shown for three different levels of the CORINE system and a fourth level from the Swedish SMD system.

forest (Table 1; Table A5). Mixed-leaved forest had a significant positive association at 300 and 800–1000 m radii buffers (Table 1; Table A5). For broad-leaved forest, there were no significant relationships with EGA at all (Table 1).

In the final model, *Level 4,* SMD (Swedish land cover classes) land cover data includes 59 land cover classes, of which 46 are represented in Stockholm County. At *level 4*, the forest classes are further subdivided and categorized depending on the forest understory. Young humid coniferous forest (not on lichen 7–15 m) show significant positive association with EGA at 200, 400–600, and 800 m radii buffers (Table 2). Older humid coniferous forest (not on lichen > 15 m), instead has a significant negative association at 700–1000 m (Table 2; Table A6). Coniferous forest on wetland, has a significant positive association at 600–1000 m radii buffers (Table 2). Dry coniferous forest (on bedrock) has a significant positive association at 600, and 800–1000 m, and on lichen rich ground at 600–800 m radii (Table 2). At *level 4* we also see

the first significant positive relation with broad-leaved forest (on bedrock), but only at 800 m radius buffers (Table 2). Limnic wetlands have a significant positive association at 200 m and the presence of lakes and ponds are significantly increasing the proportion of EGA at 200 and 400–1000 m radii buffers (Table 2).

When we analysed landscape diversity, measured as a Gini-Simpson index calculated from the proportion of all different land cover types present, we found no significant association with EGA at the smallest buffer zone with a 100 m radius (Table A7). There were several significant positive relationships between landscape diversity and EGA (increasing risk of EGA) at *level 3* and at *level 4* (Table 3; Table A7). For *level 4*, which has the highest number of land cover types, there was a significant positive association between EGA and landscape diversity from 200 to 900 m radii (Table 3; Table A7). At *level 1*, we found significant positive relationships at 200 m and 500 m radii buffers (Table 3; Table A7). At *level 2*, the only significant positive relationship
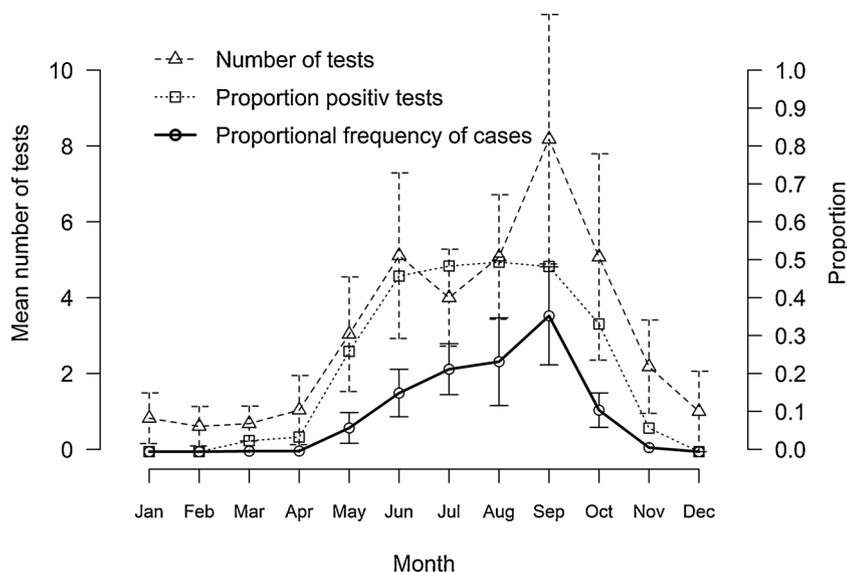
Fig. 3. The mean monthly proportion of the annual number of cases of *Anaplasma phagocytophilum* in horses in Stockholm County, Sweden for the period 2002–2015 (circles, solid line). Also shown is the mean total number of tests per month (triangles, broken line) and the mean proportion of positive tests per month (cross, dotted line). Error bars denote ± 95% CI.

Table 1

CORINE Land Cover *level 1 - 3*. Regression coefficients and significance levels for the effect of land cover types explaining Equine Granulocytic Anaplasmosis in Stockholm County. The table show all significant variables from the final generalized linear model.

		Buffer zone radius, m				
CORINE land cover level 1-3		200	400	600	800	1000
Level 1	Agriculturall areas	−1.23**	ns	ns	ns	ns
	Forest and seminatural	ns	1.70**	1.80**	1.98**	2.03**
	Water bodies	ns	3.16*	2.81**	3.16**	3.18**
Level 2	Artificial vegetated areas	2.22*	ns	8.82*	ns	ns
	Forest	1.54**	2.04**	7.23**	2.48**	2.48**
	Inland water	ns	3.43*	9.03**	4.50***	4.51***
Level 3	Coniferous forest	1.78*	ns	2.11*	2.02*	1.94*
	Mixed forest	ns	ns	ns	6.09**	7.69**
	Inland water bodies	ns	ns	4.68***	4.94***	4.55***

Significance levels: ns = P > 0.05, * P < 0.05; ** P < 0.01; *** P < 0.001.

Table 2

SMD (Swedish land cover classes). Regression coefficients and significance levels for the effect of land cover types explaining Equine Granulocytic Anaplasmosis in Stockholm County. The table show all significant variables from the final generalized linear model.

	Buffer zone radius, m				
SMD land cover level 4	200	400	600	800	1000
Dump sites	ns	ns	122.61*	137.51**	165.19**
Camping/summer houses	3.21*	ns	9.64*	9.88*	ns
Broad-leaved forest on bedrock	ns	ns	ns	169.15*	ns
Coniferous forest lichen rich ground	ns	ns	18.02*	23.34*	ns
Coniferous forest not lichen 7-15m	2.60*	2.93*	7.32*	6.10*	ns
Coniferous forest not lichen > 15M	ns	ns	ns	−47.46*	−38.91*
Coniferous forest on wetland	ns	ns	5.55*	6.35*	3.82*
Coniferous forest on bedrock	ns	ns	9.14*	9.35*	7.56*
Lakes and ponds open	6.43*	27.83*	8.01*	9.31***	5.75***

Significance levels: ns = P > 0.05, * P < 0.05; ** P < 0.01; *** P < 0.001.

Table 3

Regression coefficients and significance levels for the effect of landscape heterogeneity on Equine Granulocytic Anaplasmosis at different buffer zone sizes. Land cover heterogeneity is measured as Gini-Simpson index for each level of land cover class and analysed with general linear models.

	Buffer zone radius, m				
Land cover	200	400	600	800	1000
CORINE level 1	1.76*	ns	ns	ns	ns
CORINE level 2	1.90*	ns	ns	ns	ns
CORINE level 3	2.34**	2.38*	2.40*	ns	ns
SMD level 4	2.36**	2.85**	3.33**	2.92*	ns

Significance levels: ns = P > 0.05, * P < 0.05; ** P < 0.01; *** P < 0.001.

with landscape heterogeneity showed up at 200 m radius buffers (Table 3; Table A7),

4. Discussion

The main aim of this study was to identify temporal and spatial factors associated with Equine Granulocytic Anaplasmosis (EGA). To our knowledge, this is one of the first studies analyzing the spatial associations for clinical anaplasmosis in horses, permitting a contemporary view of *Anaplasma phagocytophilum* presence in Stockholm County.

4.1. Temporal changes

During the studied time period there was an increase of the proportion positive EGA test results. In Europe, other tick-borne diseases, in particular Lyme disease and tick-borne encephalitis (TBE), have been increasing since the 1980s (Jaenson et al., 2012; Amicizia et al., 2013). Climate change is frequently involved as an important factor in the expansion of tick-borne diseases. The change in climate is suggested to have contributed to the shifts in the northern distribution of *Ixodes ricinus* ticks, and to the general increase in tick densities in the central and southern parts of Sweden (Lindgren et al., 2000; Jaenson and Lindgren, 2011; Jore et al., 2011; Jaenson et al., 2012). Previous studies have also shown that the prevalence for *A. phagocytophilum* in horses are related to tick densities in the same areas (Hansen et al., 2010).

The active tick season in Stockholm County range from April – November. In our study EGA varied significantly across seasons, with the highest proportion positive test as well as the highest number of

PCR tests performed in late summer and early fall. A previous study from Stockholm County showed that larvae and nymph ticks peak questing activity is in June, whereas adults are mainly active in July (Asghar et al., 2016). Symptoms of EGA normally occur within 1–2 weeks of a tick bite, hence the seasonal pattern of EGA cases seem to follow the seasonal pattern of tick activity. Prevalence of *A. phagocytophilum* has also been more frequently detected in adult ticks, with a clear unimodal seasonality for infected adults with a peak in September (Reye et al., 2010; Mysterud et al., 2013; Soleng and Kjelland, 2013).

4.2. Associations with landscape features on EGA

A previous study on risk habitats for ticks suggested that different categorizations and continental scales may not present consistent results (Estrada-Peña et al., 2016). Our results indicate that the identified associations between land cover variables and EGA are robust to differences in categorizations among different classification levels. We found that the results from the four land cover classification levels were surprisingly congruent. For example, there was a significant increased risk of EGA with forest cover found at all levels. This means that it is possible to predict EGA risk patterns using crude large-scale land cover patterns, at least if we utilize CORINE Land Cover *level 2* or *3*. However, analysis at the more detailed *level 4*, showed that there are differences among forest types in how they may affect EGA with some forests even having negative associations. This indicates that to understand how different risk factors create local distribution patterns of *A. phagocytophilum,* we need to study landscape configuration in more detail.

This study also shows the importance of measuring land cover types at different buffer zone sizes around each case location. The lack of significant land cover variables in the smallest buffer zones indicate that the first 100 m only cover the actual farm. The significant relationships between EGA and the two land cover types woodlands and water bodies became apparent first when we included land cover up to 200 m, indicating that land cover types in the adjacent landscape are important to capture the larger context of habitat suitability for *A. phagocytophilum.* Tick abundance has been found to be higher at the outskirts of pastures with adjacent tree cover, and pastures that are grazed by animals all year round (Gilbert et al., 2017). At distances more than 100 m from the center of the farm pastures and forests can begin to create favorable tick habitats. It is also at these distances that we start to see negative associations (decreased risk of EGA) of agricultural areas. We interpret this as if there is arable land instead of forest close to the pastures near the farm, the risk of EGA is lowered.

For most of the land cover variables with positive associations, the impact emerged at distance between 100–300 m from the farm. In an earlier study measuring tick abundance, we found that landscape effects disappeared at 700–900 m (Asghar et al., 2016). In this study, where we are analyzing relationships between land cover types and EGA, many landscape associations stretch all the way up to 1000 m. Since large vertebrate hosts have home ranges that stretches several square kilometers, even larger buffer zones may need to be considered. This may also reflect that the buffer zones are not centered exactly at the permanent positions of the horses. During the summer, a horse spends a large part of the time in pastures that may be several hundred meters from the center of the buffer zone. One important deviation from this pattern is the positive relationship between landscape heterogeneity and EGA, which starts at 200 m for all classification levels (*level 1-4*) and ends at 900 m for the most detailed land cover classification level (*level 4*). This indicates that *A. phagocytophilum* is affected both by the border habitats of the pasture, as well as the surrounding landscape.

Analyzing several statistical models, as we did in this study, there is always a risk that some significant results will appear by chance. However, our results show several clear patterns of land cover variables that have significant relations with EGA. The most obvious result is the positive association with forest that we can see at all classification levels of land cover classes (*level 1-4*). Forests cover almost half of the

total area of Stockholm County, and woodlands are considered a natural habitat for many tick vectors and hosts since they provide shelter and a buffering effect to weather extremes like variation in temperature and humidity (Lindström and Jaenson, 2003; Tack et al., 2012). Several earlier studies have investigated habitat and environmental conditions for ticks and found that especially broad-leaved forests have high abundance of ticks (Lindström and Jaenson, 2003; Jaenson et al., 2009; Vanwambeke et al., 2016). In our study, we found positive relationships with broad leaved forest and with mixed forest, but the associations were very weak and only present at 600–800 m buffers. This is most likely due to the fact that broad-leaved and mixed forests are relatively scarce in Stockholm County. On the contrary, different coniferous forest types are very common in the landscape and showed positive relationships with EGA at most buffer zone sizes.

Using the most detailed land cover classification (*level 4*), we were able to distinguish between different types of coniferous forests. EGA was positively related to young humid coniferous forests, and to coniferous forests on wetlands. In the case of the vector, it may not be the forest type itself that makes the habitat suitable, but rather an assemblage of resources that allows for the tick to fulfil its lifecycle (Vanwambeke et al., 2016). Previous research has shown that coniferous forests with a moist litter layer, may support high tick densities (Estrada-Peña, 2001; Walker et al., 2001). Surprisingly, we found a negative association between old humid coniferous forests and EGA. One possible explanation is that since ticks thrive in dense, lower vegetation (Medlock et al., 2008; Hornok et al., 2014), old forests with dense canopies and more sparse ground vegetation may be less favourable for both ticks and their hosts. Another, simpler explanation, is that there is more young forest than old forest in Stockholm County. Therefore, horse pastures are less likely to border old forests even in landscapes with relatively high amount of old growth forest.

Our results also showed positive relationships with dry coniferous forest and coniferous forest on lichen rich ground. Since ticks essentially are sessile organisms, they depend on host movements for dispersal (Ruiz-Fons and Gilbert, 2010). Therefore, host movements and habitat usage can determine tick dispersal and pathogen presence. Habitat selection by larger mammals is best viewed as a trade-off between selection of cover and food (Mysterud et al., 1998). For roe deer, the amount of protection and shade provided by the vegetation during day-time, and distance to grazing areas may indirectly effect tick density and pathogen prevalence (Lindström and Jaenson, 2003; Ruiz-Fons and Gilbert, 2010). Dry coniferous forest, and coniferous forest on lichen rich ground are good foraging habitats for browsers like moose, but they also provide shelter for mammals like roe deer and hare. Roe deer and hares are also common foraging visitors in horse pastures, proliferating the spread of ticks and their accompanying diseases from hosts in the forest to horses.

Using the different hierarchal levels of classification found in the CORINE Land Cover system, our results indicated that closeness to water bodies increased the risk of EGA. Further, using the most detailed classification level (*level 4*) found in the SMD, we saw that specifically wetlands, and lakes and ponds showed positive associations. Stockholm County, which is located on the Baltic Sea coastline, is characterized by scattered wetlands, but there are also lots of small and large lakes. Lakes and wetlands do not strictly constitute a suitable habitat for ticks or their hosts, but they can provide the necessary humidity for the surrounding environment (Herrman and Gern, 2015). Being surrounded by large bodies of water, this area has high soil moisture, and a buffering of the temperature, which increase the vegetation period (Jensen et al., 2000; Jaenson et al., 2009).

Lastly, our study showed that EGA was positively influenced by landscape heterogeneity. There was no association between EGA and landscape heterogeneity at the smallest buffer zone size (100 m). In these small buffers most of the landcover is the farm itself classified as built area. At the next buffer zone size (200 m) there were significant positive associations with landscape heterogeneity at all classification

levels, indicating the importance of the landscape in the close vicinity of the focal pasture. Important to remember is that a positive association means more EGA in heterogeneous landscapes. The positive relation with landscape heterogeneity was present also at larger buffer zones for *level 3* and *4*, but not for *level 1* and *2*. This may indicate that the coarser classifications found in the Corine Land Cover *level 1* and *2* are not sufficient to indicate associations with landscape heterogeneity. The smaller buffer zone which is close to the farm is likely to include both agricultural and forest categories. The larger buffer zones are more likely to be dominated by the land cover category "forest" than the smaller buffer zone. Therefore, a higher resolution in the form of more detailed categorization, including different coniferous forest types, is necessary to be able to detect associations with heterogeneity in the adjacent landscape at distances above 200 m from the farm.

In general, small and fragmented landscapes are unfavourable to specialist species, but can be favourable to generalists. Therefore, heterogeneous landscapes can be favourable for a bridge vector like *I. ricinus* since they create a mixture of patches with suitable habitats for several hosts. Halos et al. (2010) found that forest fragmentation is linked to patch surface changes, which increases the amount of forest edge. The vegetation complexity is greater at these edges because of the mix of landscape elements, which increases the abundance of small vertebrate hosts. The forest edge communities are important for many mammals altering competitive hierarchies among different species. Species of mice and voles, two common hosts for ticks, have different distribution patterns with mice being the dominant species in the forest and voles in the open adjacent fields. The forest edge is the ecotone where the species groups are meeting at equal grounds (Manson et al., 1999). Therefore, the edge may once again act as a link for ticks traversing from the forest and forest edge out into the pastures. Small fragments of wooded habitats can also be important in maintaining landscape connectivity and host diversity (Perez et al., 2016).

In Stockholm County, the majority of farms and stables are located in urban and peri-urban areas (Enhäll, 2017). In these areas, landscape changes, management practices or creation of recreational parks may facilitate tick-host populations and increase the exposure to ticks (Beugnet and Marié, 2009). Certainly, additional factors are relevant in the temporal and spatial distribution, for example climatic variability (Grey et al., 2009; Qviller et al., 2014). However, the current study indicates the significant role of land cover types and habitat fragmentation, variables that are amenable to management. These results will help to modify land cover guidelines and recommendations based on the different classification levels of the land cover variables.

5. Conclusions

Our results show the complexities of the parasite-host-habitat relationship. This study suggests that young humid coniferous forests, water bodies and heterogeneous landscapes increase the risk for EGA. We also found that CORINE and SMD classification levels (*level 1-4*) give congruent results when analysing single habitat types, and that landscape configuration can give important indications for disease presence. However, in a landscape dominated by coniferous forest, we needed a high resolution of the landscape data to be able to detect associations between landscape heterogeneity and EGA at larger distances from the focal pasture.

The information on the eco-epidemiological drivers for EGA can be central for disease control and prevention and may also be helpful in understanding the ecology of other tick-borne diseases like Lyme disease and Tick-borne encephalitis (TBE). The results from this study may also be significant for the medical health field, since the agent causing Equine Granulocytic Anaplasmosis in horses is identical to the agent causing Human Granulocytic Anaplasmosis (HGA) (Egenvall et al., 2001).

Our study approach linking land cover to infectious disease risk tries to tackle the difficulty of relating a low-prevalence disease to landscape

factors. Our GIS indicators can be applied to other tick-borne diseases and animals or humans at risk of contact with ticks. The accuracy could be improved with a better surveillance scheme, with an annual census of the horse population, a precise case definition for the referring veterinarians and shared laboratory protocol between laboratories. While the conditions for suitable habitats for *A. phagocytophilum* vectors may be specific to Scandinavia, the easily accessible CORINE landscape descriptors offer interesting perspectives for further explanations for various tick-borne diseases in different habitats.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2019.106626.

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