THE EMERGENCE AND SPREAD OF TICK-BORNE ENCEPHALITIS VIRUS IN SCANDINAVIA

Ola Hall[a]
Helena Eriksson[a]
Peter Nolskog[b]
Tomas Bergström[c]
[a] Department of Earth and Ecosystem Sciences
Sölvegatan 12, 223 62 Lund, Sweden
[b] Department of Infectious Diseases, Skövde Hospital, Skövde, Sweden
[c] Department of Infectious Diseases, section for Clinical Virology, University of Göteborg, Sweden
Ola.hall@nateko.lu.se,

ABSTRACT

Tick-borne encephalitis virus (TBEV) is a high-impact pathogen with approximately 12,000 diagnoses annually in Eurasia. The virus causes a variety of clinical manifestations, with neurological symptoms in up to 30% of the patients. Lethality in Europe is <2% but post-encephalitis syndrome is seen in over 40% of the infected patients and results in substantial impairment in quality of life. Over the last decade a drastic increase in TBEV incidence has been reported throughout Western Europe

TBEV is a member of the Flaviviridae virus family, which are primarily spread through arthropod vectors (mainly ticks and mosquitoes). At some point during their transmission cycle, nearly all vector-borne diseases are linked in some way to a vegetated environment. This makes them suitable objects to study with remotely sensed multi-spectral data. In fact, remote sensing is becoming an important tool in active disease surveillance. Evidence of disease is monitored in endemic areas, which could help prevent outbreaks or slow transmission in the early stage of an epidemic. Applications of remote sensing in epidemiology involve retrieving environmental variables that characterize the vector ecosystem (land cover, temperature, humidity, water pressure, precipitation).

In this study we report on a discriminant analysis of remotely sensed vegetation indices applied to TBEV positive sites and to TBEV negative sites. Results indicate that there is a difference between sites, indicating a relationship between ticks with TBEV and certain environmental characteristics.

INTRODUCTION

The incidence of tick-borne encephalitis (TBE) in Sweden and Western Europe has increased since the mid-1980s (Lindgren et al. 2001). For a long time, TBE was endemic in the Stockholm region, though in recent years we have seen it spread north and south along the Baltic sea coast, but the most dramatic spread has been, south west, to the Western Gotaland region (Figure 1). The increase in TBE cases and observed changes in geographical distribution remains mostly unexplained. Milder winters due to climate change, new recreational habits, changes in landscape structure and pattern are some of the explanations put forward by the research community. The low-endemic region of Western Gotaland region is well suited for studies of the epidemiology of TBE virus and provides unique possibilities to answer basic questions regarding TBE and environmental variables. Nearly all vector-borne diseases show some association with a vegetated environment during some phases of their transmission cycle. This makes them suitable objects to study with remotely sensed multispectral data.

The aim of this paper is twofold: 1) to provide a review on the TBEV situation in Europe in general and Sweden in particular. Here, we address *I. ricinus* distribution and ecology in particular, as well as contemporary explanations for the upsurge and spread of TBEV. 2) to test if there exists a difference in the physiographic conditions between sites which harbor TBEV-infected ticks and sites where only non-infected ticks are found. Here, we applied Landsat-5 derived vegetation indices to approximately 200 sites and performed a discriminant analysis.

Ixodes ricinus

The TBEV is a member of the genus flavivirus and belongs to the family of Flaviviridae, members of which are primarily spread through arthropod vectors (mainly ticks and mosquitoes). There are three subtypes of TBEV: the European (TBEV-Eu, transmitted by *Ixodes ricinus* ticks) and the Siberian and the Far-Eastern subtypes (TBEV-Sib and TBEV-FE, transmitted by *I. Persulcatus* ticks) (Ecker et al. 1999; Mavtchoutko et al. 2000; Lundkvist et al. 2001). TBE-Eu strains are very similar and strains within countries, do not form distinct genetic sub-groups, and have begun to spread perhaps only in the last few centuries. The other two subtypes, carried by *I. persulcatus*, show much greater diversity and have probably been evolving for thousands of years (Lindquist et al. 2008).

The common tick, *Ixodes ricinus*, is the primary European contagion vector to humans of the pathogens *Borrelia afzelii, B. burgdorferi s.s* and *B. garinii, which* cause tick-borne encephalitis (Nuttall et al. 1994). On a global scale, only mosquitoes are considered to be a medically more important group of arthropods (Lindström et al. 2003). *I. ricinus* is the most common tick species of the eleven species permanently present in the Nordic countries. More than 95% of all ticks found on humans, dogs, cats, horses, moose, etc. belongs to the specie *I. ricinus*.

The geographical distribution of *I. ricinus* extends from northern Africa to northern Europe. *I. ricinus* is seen in most of Europe, and the distribution extends to Turkey, northern Iran, and Caucasus in the Southeast (Lindquist and Vapalahti 2008). In the Nordic countries *I. ricinus* is common in the Danish islands, the southern parts of Sweden, extending north to Värmland, and along the coast of Norrland. In Norway the tick is mainly found in the southern and western regions, extending along the coast to Nordland. *I. ricinus* is common in the south of Finland also. TBEV is not found throughout the entire spatial distribution of *I. ricinus* populations (unlike *Borrelia burgdorferi*), indicating a strong dependence of additional environmental factors.

There are four developmental stages in the life cycle of the tick: eggs, larvae, nymph, and adult. The period needed to mature from the larval to adult stages is approximately 2-6 years, but typically 3 years. At each stage ticks must consume blood to develop into next stage, feeding on any suitable host present. Viral transmission to un-infected ticks occurs when ticks at different development stages co-feed on the same animal. In the developmental stages of *I. ricinus,* nymphs are most abundant and least host specific, and therefore most important in viral transmission to humans (Süss 2003). Occasionally, the virus is transmitted transovarially, e.g. from an infected female to egg, or from viraemic animals.

The presence of hosts and a suitable habitat with adequate vegetation cover are important for the survival and development of the tick (Lindström and Jaenson 2003). Elevation, temperature, rainfall, and humidity are key abiotic factors that influence the presence, development, activity, and longevity of the tick. For locations in Spain, Estrada-Peña (Estrada-Peña 2001) reported the highest abundance of *I. ricinus* in oak (*Quercus*) and mixed old forests with many ecotones. Ticks were absent from open biotopes. Ticks seemed to prefer sites with secondary plant growth (river canopies, mixed forests, deciduous heterogeneous woods). *I. ricinus* was absent from open biotopes, homogenous young coniferous forests and open hillsides. In Scotland, it was found that coniferous woodlands showed the highest infestation with questing I. ricinus nymphs, deciduous woodlands had slightly lower infestation levels, and the upland pastures had much lower infestation (Walker et al. 2001). This study also reported that *Vaccinum myrtillus/Calluna vulgaris* vegetation showed the highest infestation, followed by *Agrostis/Festuca/Holcus* grassland, and then *Pterdium* vegetation, with the least tick abundance.

Mejlon and Jaenson (Mejlon et al. 1993) investigated the abundance of ticks in different biotopes in south-central and southern Sweden. Mixed spruce/deciduous woodland had the greatest nymphal abundance, followed by spruce forest, whilst cattle pasture showed the least abundance. In a study comprising 16 European countries and related to *B. burgdorferi* s.l., Gray et al. (Gray et al. 1998) found that large numbers of infected nymphs occurred in heterogeneous woodlands with a diverse fauna, usually including cervids. In the Österlen region of south Sweden, the greatest abundance of ticks was found in mixed deciduous, alder, oak, hazel, and beech woodlands, while open grassed areas with dry meadow and heath supported smaller populations (Lindström and Jaenson 2003).

Climate is regarded as the principal restricting factor for the northern limit of *I. ricinus* extent (Jaenson et al. 1994). The tick is very cold-hardy and can, once winter-acclimatized, endure 24-hours periods with temperatures -14° C to -20° C but temperatures below freezing can be lethal when the exposure lasts for several weeks, particularly for unfed nymphs and diapausing engorged larvae and nymphs (Dautel et al. 1997). Moltings ticks are even more vulnerable so if summer temperatures are not high enough to allow complete maturation into adulthood before the onset of winter, they are unlikely to survive even moderate exposure to frosts.

The expansion of the northern limit of *I. ricinus* in Sweden is related to climatic changes seen over several seasons where the number of degree-days showing temperature conditions favorable for tick survival, activity and development has increased (Lindgren et al., 2000). Similarly, it has been shown that, in areas with medium-high densities of ticks, increases in tick abundance were correlated with both mild winters and extended spring and autumn seasons (Lindgren and Gustafson 2001). This observation correlated well with increased TBE incidence for

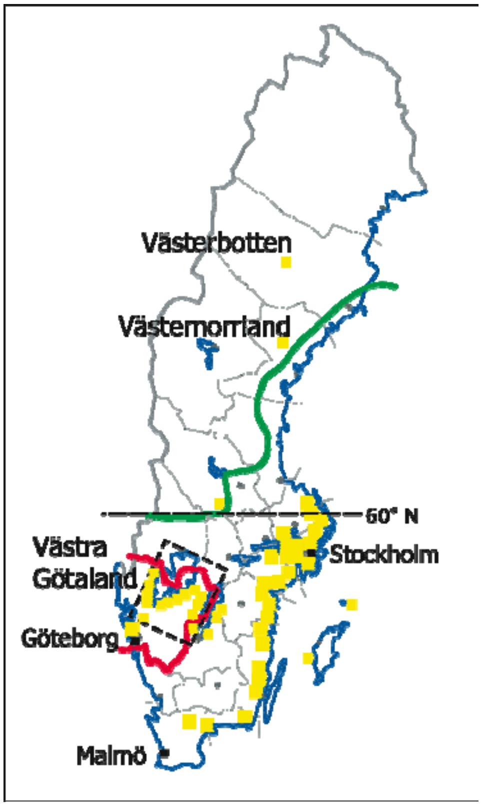

Figure 1. Map of Sweden illustrating sites discussed in the text. Green line= estimated northern continuous distribution of I. ricinus according to Jaenson et al.(2009). Red line= boundary of Western Gotaland region. Black bounding box= study area with the two major lakes, Vänern and Vättern. Yellow= TBE distribution. Note: TBE cases are mostly found near water bodies.

the same period.

TBEV Increase and Geography

TBE incidence rates and tick distribution are not uniformly distributed over the spatial distribution of *I. ricinus*. TBE incidence increased 2- and 30-fold in Central and Eastern European countries in the early 1990s, while in Western European countries the increase was more gradual. The Czech Republic experienced a sudden increase in TBE cases in 2006 (Daniel et al. 2008). This one year epidemic was explained by unexceptional weather conditions, with a warm and rainy spring, an early summer, and a cool and rainy August. High host seeking activity in *I. ricinus* during the peak summer period triggered increased outdoor recreational behavior, for example fewer people were prepared to venture into wooded areas for mushroom foraying.

In the early 1990s, the Baltic countries and Poland experienced more dramatic increases in national TBE incidence than anywhere else in Europe (Sumilo et al. 2007). Annual incidences increased from 0.5-7 to 11-54 cases per 100,000 people. With the exception of Slovenia, these are the highest levels recorded in Europe. The pattern of TBE increase in individual reporting units was non-uniform and typically extremely sudden. Furthermore, the increase was not correlated with previous high-risk conditions.

The incidence of TBE in Sweden has substantially increased since the mid-1980s. TBE incidence was compared TBE incidence rates data with data for the number of days per season where temperatures reached or exceeded thresholds important for tick persistence and pathogen transmission (Lindgren and Gustafson 2001). Findings indicated that the increase in TBE was related to changes towards milder winters and the early arrival of spring during this period.

Changes in surveillance and diagnostic practices are sometimes used to explain the upsurge in TBE cases, but this theory can be forcefully rejected in countries with long histories of TBE surveillance, e.g. Sweden and the Czech Republic. Similarly, Sumilo showed convincingly that the observed heterogeneity in timing and degree of changes in the incidence data from the Baltic countries cannot be explained in terms of changes in surveillance programs or in national public health policy (Sumilo, Asokliene *et al.* 2007).

There is yet no valid explanation for the observed rapid increase in the incidence of tick-borne encephalitis seen over the past two decades in many parts of Europe. Climate change is often a proposed cause of the observed upsurges, not only TBE, but of many different tropical and temperate vector-borne diseases.

The western-type TBEV transmission seems to depend on specific climatic conditions supporting a synchronization of activities between larvae and nymphs., It has been consistently shown that, particularly at TBEV foci sites, larvae start feeding and questing earlier in the year, and that their appearance in the spring coincides with dates when nymphs first become active (Randolph et al. 2000). The larvae-nymph synchrony was also associated with a rapid fall in ground-level temperature seen between August and October. There are indications that reduced biodiversity could also lead to increase viral transmission in favorable hosts (Dobson et al. 2006).

The TBE virus transmission route demands that larvae and nymphs feed together on individual rodents (co-feeding), and co-feeding occurs to a different degree depending on the temperature-driven patterns of seasonal activity which are important for each stage of tick development (Sumilo, Asokliene et al. 2007). The co-habitation of ticks with vertebrate hosts is not sufficient for TBEV circulation. The missing ingredient seems to be the synchronization of the seasonal activities of larval and nymphal ticks and it is this which permits viral infections to be transmitted from infected nymphs to a large numbers of infectible larvae. The seasonal synchrony hypothesis predicts that TBEV foci are characterized by climatic conditions that promote a particular pattern of *I. ricinus* seasonal dynamics.

The patchy patterns of TBEV foci are inconsistent with the idea of climate change as the sole determinant for the increase in TBE incidence. Climatic conditions are similar throughout the Baltic region and are too homogenous to account for the heterogeneous pattern of change in TBE incidence (Sumilo, Asokliene et al. 2007). Human habits strongly influence disease incidence and seasonal distribution. Tick activity is usually at its peak during leisure time in Europe. Some authorities explain the increase of TBE cases in terms of previous under-diagnosis and increased attention through newly established surveillance programs. However, increases in TBE incidence are also seen in countries with well-established diagnostic routines and long-term surveillance programs (e.g. Sweden and the Czech Republic), strengthening the argument in favor of measurable increases in TBE incidence. Other studies relate the TBE increases to cultural factors, e.g. change in recreational behavior (Daniel et al. 2008). Recent findings indicate that TBEV transmission in Russia is related to the first land road into Siberia and the Trans-Siberian Way that functioned at different times. The main reason for such rapid distribution of the S-TBEV strains is the anthropogenic factor, i.e. human economic activity during the colonization of new territories in Siberia in recent times (Kovalev et al. 2009).

NEW ENDEMIC FOCI

During the last few decades, both new endemic foci and an increase in cases have been reported in Europe. The first reports on TBE from Scandinavia date back to 1954 (Sweden), 1956 (Finland), 1963 (Denmark) and 1997 (Norway) (Skarpaas et al. 2006). In Sweden, TBE has been under routine surveillance since 1956 and most TBE natural foci were placed around Stockholm. Recent years have seen a spread in TBE and it can now be found along most of the east coast of Sweden from Uppland in the north to Blekinge in the south, around a few lakes in Skåne, and most recently, TBE has spread into the western Gotaland region, around lakes Vänern and Vättern. In addition, isolated cases have been diagnosed as far north as Västerbotten (2008) and Västernorrland (2009) (See figure 1).

TBE is an emerging disease in the western Gotaland region and the first local case was diagnosed in 1997. The epidemiological pattern was repeatable as index cases were followed by new cases in the absolute geographical vicinity (Bergström T et al. 2009). Now, >20 cases/year are reported and the TBEV is the most threatening of the emerging diseases in this region. In addition to routine surveillance of TBE cases and seroprevalence studies, Brinkley (Brinkley et al. 2008) performed a study in free-living tick populations sampled from the Western Gotaland region with the aim of determining TBEV prevalence and quantities of TBE RNA. According to expectations, they found that TBEV prevalence was low for these newly developed TBEV foci (0.10—0.42%). Unexpectedly, they found that prevalence data for these newer I. ricinus populations were comparable with data from other countries with more established endemic regions, indicating a unpredicted rapidity and magnitude with which TBEV foci may have become established. Furthermore, phylogenetic analysis found that the Western Gotaland strain (Vinninga) is of the Western subtype, and has most likely spread from Central Europe up into Sweden and then into Norway , as opposed to a spread of the Eastern or Siberian virus from Russia and Finland.

Discriminant Analysis of TBEV Locations

The Western Gotaland region provides a unique opportunity for detailed studies of the emergence and development of new TBE foci. To date, there is no solid explanation for the appearance of TBEV in the region. TBEV in ticks is only found 1) close to water bodies and 2) where TBE cases are localized. In fact, the emergence of TBEV and the observed geographical pattern of TBE cases in Western Gotaland cannot be predicted by typical epidemiological models (Bergström et al. 2009). It is puzzling, that one bay of lake Vänern may be hit by a multitude of TBE cases, while a similar neighboring area is completely spared.

This provides an opportunity to investigate if there is a difference in physiography between sites with confirmed TBEV and other locations without TBEV. Here, we report on a pilot study in the Western Gotaland region where satellite derived vegetation indices are used to characterize TBEV sites.

Data and Methods

The study area is located in the south west part of Sweden, covering most of Western Gotaland, about 57-59°N, 11-14°E, fig. 1. The climate is mild relative to the high latitude, with yearly mean temperatures ranging between about 7.7° C. (coastal areas) and 5.1° C. (inner land areas). Seasonal variations are high, with mean temperatures for July being about 16°C., and 0° C for January. Western Gotaland holds 9 000 km. of water courses and about 4000 lakes of a size larger than 0.01 km^2. The largest lake in Sweden, Vänern (5.6000 km^2), is located in the middle of the study area, with the north-eastern border demarked by Sweden's second lake, Vättern (1.800 km^2). The landscape is rather plain along the southern border of lake Vänern (elevations about 20-40 amsl), the south-eastern and north-western parts being more hilly, with elevations up to 300 amsl (Helmfrid 1996). Precipitation varies extensively within the area, from about 550 mm annually? in the lowlands to about 1000 mm (units?) in the more hilly areas towards the south (Frizell and Werner, 2003). The most common wind directions range from W and S, with yearly mean wind speeds between 2.5 to 7 m/s, the higher speeds found along the west coast in Gotaland. The extent and longevity of the snow cover varies within the area, from about 100 days of coverage in the hilly south eastern parts, to only 40 days around the coastal areas. Vegetation is dominated by planted coniferous and mixed forests, Norway spruce (Picea abies (L.) Karst.) and Scots pine (Pinus sylvestris L.) being the dominant species. Minor proportions of deciduous forests are also found, with oak, beech, birch and broadleaf forest species. The agricultural land takes up about 25% of the land area, and pasture land about 3-4%.

All TBE cases in the Western Gotaland region have been followed up since the outbreak in 1997. Through in-depth interviews with TBE patients, the exact exposure sites, rather than residential address, are known for most TBE cases. Ticks collected in TBEV-positive locations, as well as in control locations, have been collected continuously since 2004 and tested for TBEV (Brinkley, Nolskog et al. 2008). This database was made available for the present project and environmental variables were sampled from 64 sites, from the period of outbreak until 2006. Another 140 randomly distributed sample sites were used as training data and for controls. They were all considered as being negative for TBEV.

The satellite data used in this study was a Landsat-5 image from August 24, 2007. A radiometric correction and computation of at-satellite reflectances was done according to the methodology in Markham and Barker (Markham et al. 1987) using calibration values from Chander and Markham (Chander et al. 2003). Parts of the satellite image were affected by cloud shadows, but no plots were chosen in these areas. The study area is generally flat, and no topographic correction was made to the data. Reflectances from bands 3 (0.63-0.69 цm), 4 (0.76-0.90 цm), 5 (1.55-1.75 цm), and 7 (2.08-2.35 цm) were extracted for further analyses, as well as the thermal band 6 (10.4-12.5 цm), which was converted to effective at-satellite temperature. Mean at-satellite spectral reflectances were computed from the pixels covering the sample plots.

Vegetation Indices

Six vegetation indices were selected for use in this study, Table I. These indices were chosen because: 1, they have been derived in such as way as to take into consideration the form of the spectra for organic matter, 2, they have previously been used for correlation with vegetation density in numerous studies (Sellers 1989; Spanner et al. 1990; Turner et al. 1999), and 3, they have proved sensitive to variations of forest densities of similar character in former studies (Chen et al. 1997; Eklundh et al. 2001; Eklundh et al. 2003; Stenberg et al. 2004).

Table I. Vegetation indices used in the study

Index	**Equation**	***Reference***
imple Ratio (SR)	$SR = \frac{TM4}{TM3}$	*(Jordan 1969)*
ormal Difference Vegetation Index (NDVI)	$NDVI = \frac{TM4 - TM3}{TM4 + TM3}$	*(Rouse 1974)*
ormal Difference Vegetation index with TM7 instead of TM3 ($NDVI_{4,7}$)	$NDVI_{4,7} = \frac{TM4 - TM7}{TM4 + TM7}$	*(Kaufman et al. 1997)*
Normal Difference Vegetation Index + MIR ($NDVI_c$)	$NDVIc = \frac{TM4 - TM3}{TM4 + TM3}\left(1 - \frac{TM5 - TM5_{\min}}{TM5_{\max} - TM5_{\min}}\right)$	*(Nemani et al. 1993)*
educed Simple Ratio (RSR)	$RSR = \frac{TM4}{TM3}\left(1 - \frac{TM5 - TM5_{\min}}{TM5_{\max} - TM5_{\min}}\right)$	*(Brown et al. 2000)*
Moisture Stress Index (MSI)	$MSI = \frac{TM5}{TM4}$	*(Vogelmann 1990)*

The SR is one of the simplest vegetation indices. It has been shown to correlate well with vegetation density in numerous studies (Chen et al. 1996). The NDVI is also very common, and many studies have found good agreement between NDVI and vegetation density (Baret et al. 1991; Nemani, Pierce *et al.* 1993; Chen et al. 2002), but a saturating effect of the NDVI has been observed for dense forests (Sellers 1989). Attempts have been made to include the middle infrared (MIR) wavelengths, there being the MSI, RSR and NDVIc. Indices including MIR have been found to correlate better to the vegetation density of temperate and boreal forests (Nilson et al. 1999; Brown, Chen *et al.* 2000; Chen, Pavlic *et al.* 2002; Eklundh, Hall *et al.* 2003). Reflectances in the MIR wavelengths decrease with increased leaf area, as a consequence of increased absorption due to water in the canopies. Therefore, the MSI index has been used to detect both the density of biomass and water content. The RSR and NDVIc indices also incorporate the minimum and maximum reflectances from MIR. These values were determined from pixels that cover the forested areas in the image.

Discriminant analysis (DA) is used to classify cases into the values of a categorical dependent, usually a dichotomy. DA can be used to test theories by observing whether cases show predicted classifications, determined though the investigation of differences between sample groups. But DA can also be used in prediction. DA was used in this study with positive and negative TBEV sites as our categorical dependent variables. As our predictors, or discriminating variables, all 7 indices (including thermal band 6) were included in the model.

RESULTS

From the analysis of the scatterplot-matrix (Fig. 2) two observations are made. 1) It is not possible to distinguish any categories from the visual inspection of scatterplots. 2) It is noted that discriminating variable histograms have distributions of varying shape. However, DA is relatively robust, even where there are only modest violations of homogeneity of variance and in the normal distribution (Lachenbruch et al. 1979). Dichotomous variables often violate multivariate normality and are not likely to affect conclusions based on DA (Klecka 1980).

Wilks' lambda (λ) is used to test the significance of the discriminant function as a whole. In this study , λ= 0.945 and p= 0.142. A significant λ means one can reject the null hypothesis that the two categories have the same mean discriminant function score, permitting the conclusion that the model is discriminating.

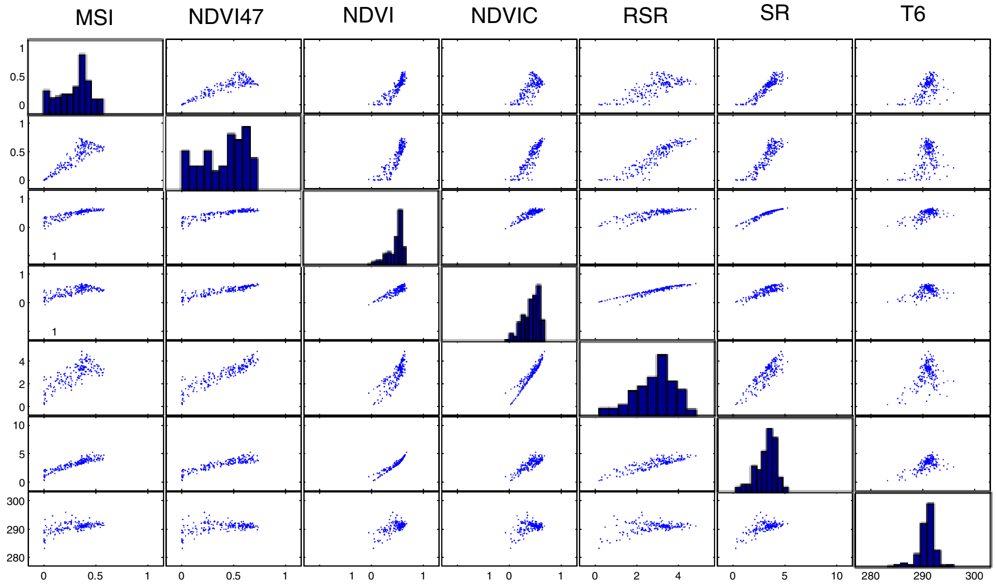

Figure 2. Scatterplot-matrix with histogram plots for all discriminating variables.

The result from the DA is summarized in table II. A confusion matrix contains information about actual and predicted classifications obtained using the selected classification system (i.e. DA). Performance of such systems is commonly evaluated using the data in the matrix. The data in table II was used to assess how well the discriminant function works, and if it works equally well for each group of the dependent variable. Here it correctly classifies about two-thirds of the cases. The overall classification accuracy is derived from adding the diagonal values and dividing by the total number of samples (33+95)/199=64.3. The model correctly predicts 33 sites as true positive and 95 sites as true negative. In addition, the model incorrectly predicts 26 sites as false positive and 45 sites as false negative. The *true positive* rate is 0.70 and is the proportion of positive cases that were correctly identified. The *false positive* rate was 0.44 and is the proportion of negative cases that were incorrectly classified as positive. The *true negative* rate was 0.56 and is the proportion of negative cases that were classified correctly. The *false negative* rate was 0.33 and is the proportion of positive cases that were incorrectly classified as negative. Finally, the *precision* rate was 0.78 represents the proportion of the predicted positive cases that were correct.

Table II. Classification result

		Predicted membership		
	Neg	45	95	140
%	Pos	55.93	44.07	100.0
	Neg	32.14	67.86	100.0
	Total	78	121	199
		39.20	60.80	100.0

If there were two or more discriminant functions they could be visualized as scatterplots showing the relation of the two first discriminant functions. The dependent variable in this study has only one discriminant function and can thereby be visualized as bar charts (Figure 3). For a good discriminant function, the bar chart will have most data points near the mean, with small tails.

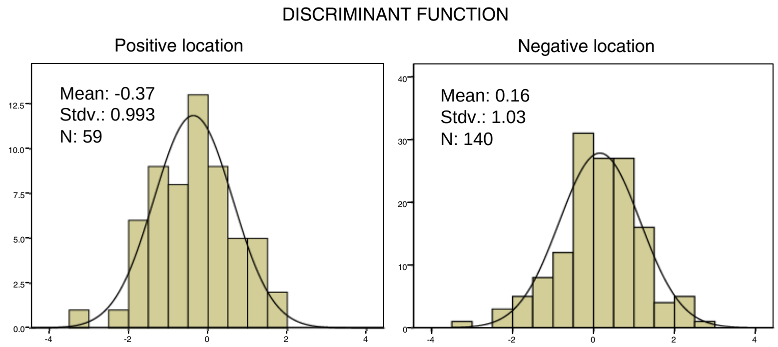

Figure 3. Discriminant function. As the dependent in this study has only one discriminant function, bar charts are displayed instead of scatterplots.

The correlation between the DA-function was tested and discriminating variables were ranked according to absolute size of correlation within the function. Normal difference vegetation index + MIR ($NDVI_c$) had the highest correlation followed by $NDVI_{4,7}$, NDVI, T6, RSR, MSI, and SR. Correlations ranged from 0.483 ($NDVI_c$) to 0.235 (SR).

DISCUSSION

The transmission route of TBEV is dependent on larvae and nymphs simultaneously feeding on the same animals. Tick survival from one stage to another depends on environmental factors such as habitat structure, moisture conditions and host availability. Together, these factors determine the focal distribution of TBEV (Sumilo, Asokliene et al. 2007).

I. ricinus is the main vector for TBEV and there is evidence that the distribution of the tick is presently extending into new territories. For example, the tick can now be found along the northern coasts of Sweden and the first cases of TBEV were recently reported. The intriguing part is that TBEV is not found throughout the spatial distribution of *I. ricinus* populations, indicating a strong dependence of additional environmental factors that restrict its presence to fragmented foci of only a few square meters to several square kilometers in size. Explanations have been put forward that probably migrating birds are responsible for the dispersal of TBEV-infected ticks (Waldenstrom et al. 2007).

Based on the review at the beginning of this paper, a few conclusions are made. We now have a good understanding of where ticks reside and develop. They are mostly absent in open biotopes and seemed to prefer sites with secondary plant growth. It is plausible that TBEV survives in tick populations through some optimization of conditions. Several authors discuss climate-induced factors as the key to sustaining TBEV and hosts. We can conclude that there is no good explanation for the patchy endemic foci of TBE.

For this study a database with detailed records on TBE infection sites was made available. From interviews with patients infected with TBEV, the exact locations of where they were bitten has been derived. A pilot study was designed with the aim of testing whether Landsat TM-derived vegetation, moisture, and temperature indices could be used to discriminate between positive TBEV sites and negative sites. Previous studies have related, for example, land surface temperatures, derived from NOAA data, with an approximate resolution of 1 km x 1 km data to TBEV sites (Randolph, Green et al. 2000). Here, we tested if the superior resolution of Landsat TM could be used to analyze the small TBEV-positive patches found in the study area.

Six of the more common and useful satellite derived indices for vegetation analysis were applied to the Landsat scene after radiometric correction and at-satellite reflectance calibrations has been performed. The positive and randomly collected negative sites were used as categorical dependents, and the indices including the thermal band 6 were used as our predictor variables. It was found that $NDVI_c$ had the best correlation (0.483) with the discriminating function. Based on correlations, variants of NDVI were superior to moisture stress and temperature to discriminate between sites.

The overall classification accuracy is relatively low (64%), indicating that the model has some capability in discriminating between different sites. An examination of the precision statistics shows that the model performs slightly better (0.78); 78% of the predicted positive cases are correct. With the relatively high spatial resolution of Landsat-5 (30 m x 30 m), there is a good chance that a TBEV site is tainted with pixels that have a membership in the other category. From visual inspection of the scatteplot matrix it is concluded that discriminating between these sites are not a trivial classification problem.

The results from this study indicate that there are certain vegetation structures that are favored by TBEV. We can also conclude that the discriminant function used in this study has too low a precision to be of predictive use. The preferred habitats of *I. ricinus* (woodlands, secondary plant growth) are particularly difficult to classify by ordinary techniques.

Future studies should include a careful examination of the relationship between observational scale (spatial resolution) and process scale (tick ecosystem scale). The complicated vertical structure of woodlands would probably benefit from a combination of spectral data and radar observations. The penetrating capabilities of radar could contribute to a better characterization of ground vegetation covered by tree-crowns.

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