NORTH-WESTERN JOURNAL OF ZOOLOGY 14 (1): 25-29
Article No.: e171301

*http://biozoojournals.ro/nwjz/index.html*

# Modeling the distribution of *Ixodes ricinus* in Romania

Cristian DOMŞA, Andrei D. MIHALCA and Attila D. SÁNDOR*

Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur str. 3-5, RO-400372, Cluj, Romania.
*Corresponding author, A.D. Sándor, Tel. +40 264 596384; Fax: +40 264 593792; E-mail: attila.sandor@usamvcluj.ro

Received: 01. November 2015 / Accepted: 20. February 2017 / Available online: 24. March 2017 / Printed: June 2018

**Abstract.** *Ixodes ricinus* is the most common and widely distributed tick species in Europe and is responsible for the most pathogens vectored. It is distributed all over in Romania, being an important vector of Tick-Borne Enchephalitis Virus (TBEV) and Lyme-borreliosis pathogens in the country. The aim of this paper was to assess the general risk associated with the presence of *I. ricinus* throughout Romania, by modeling the distribution based on known presence location and environmental variables. To model the distribution we used the maximum entropy approach, using presence only records (246 individual locations) and environmental variables. WorldClim provided high resolution interpolated climate data rasters were used, together with altitude and forest cover percentage of any individual cell. The model was fitted using MaxEnt, following a testing of the 75% threshold of occurrence records to construct the model. The value for AUC was 0.724 (for test data), indicating a good performance for our model, the minimum training presence threshold provided by the model was 0.076 (used to calculate the climatically suitable area). The variable with the largest contribution to the model were forest cover (49.7%), followed by annual precipitation amount (16.2%). Using a probability threshold value of 0.5 for elevated risk of encounter, the 5 counties in the central part of Romania showed as high probability presence areas. These areas also host the highest incidence of TBEV and Lyme-borreliosis cases reported, together with highest diversity of selected pathogens, thus suggesting a good predictive power for the model we selected.

**Key words:** *Ixodes ricinus*, maximum entropy modeling, risk map, ticks distribution, Romania.

## Introduction

The distribution of tick-borne diseases is directly correlated with the distribution of tick vectors as well as the vertebrate reservoirs of the pathogens. The geographical distribution of ticks is normally assessed by field collection of ticks from the environment as well as from hosts. *Ixodes ricinus* is considered to be among the most widely distributed and most abundant tick across large areas of Europe and among the most important tick-vectors for a wide diversity of human and animal pathogens (Rizzoli et al. 2014). Knowledge of its distribution has greatly expanded during the last decades, being attributed to several factors, including land use and land cover, climate change, and changes in the abundance and community structure of wild hosts (Rizzoli et al. 2014). Considering that *I. ricinus* is a very generalist tick species (i.e. with no host specificity) and that, as most *Ixodes* species, it spends ca. 95% of its life off-host (Ostfeld & Brunner 2015), its distribution is correlated mostly with abiotic factors.

Recent studies in Romania have shown that *I. ricinus* is the most common tick species, widely distributed in most habitats of the country (Mihalca et al. 2012a, Sándor et al. 2017) and the most common tick feeding on humans (Briciu et al. 2011). All these aspects make *I. ricinus* a good candidate for spatial modeling studies.

*I. ricinus* is the most important vector for zoonotic pathogens in Europe, known to play a crucial role in the distribution and incidence of Lyme borreliosis, tick-borne encephalitis or human granulocytic anaplasmosis, to name just a few (for a full checklist see Briciu et al. 2011). Moreover, single individuals of this species were recorded to simultaneously harbor several pathogens, resulting in so called co-infections (for details, see Rizzoli et al. 2014). In most cases pathogens are acquired from zoonotic vectors through larvae feeding on small mammalian hosts (rodents, insectivores) and transmitted through all development stages, primarily by nymphs (Mihalca & Sándor 2013, van Duijvendijk et al. 2015). While vertical transmission rates are low (<1%), the number of individual ticks (usually determined by egg-laying females) in a certain geographical area is the most important predictor for epidemiological risk (Coipan et al. 2013). Thus, by building a powerful model, one may locate those geographical areas which pose the highest epidemiological risk in a defined geographical area. While general distribution of the species is known and recent studies project a continuous growing of its geographical distribution, both altitudinally (Materna et al. 2005, Martello et al. 2014) and latitudinally (Jaenson et al. 2012, Jore et al. 2014) all over Europe, there is no local or regional risk-map created for this species in Romania. The aim of this paper is to assess the general risk associated with the presence of *I. ricinus* throughout Romania, by modeling the distribution based on known presence location and environmental variables.

## Materials and methods

### Ticks distribution data

To model the distribution of *I. ricinus*, we used the maximum entropy approach (Phillips et al. 2006, Porretta et al. 2013, Signorini et al. 2014). Tick distribution data used for this study were compiled both from published literature (Mihalca et al. 2012a, b) and field data obtained by flagging (dragging a cloth on vegetation and collecting the seeking ticks mechanically attached to it) (see Fig. 1 for the distribution of records). A total of 11,965 *I. ricinus* ticks were collected from 180 locations in all 41 counties of Romania in the years 2009-2011 (Mihalca et al. 2012a). Flagging has been performed in randomly chosen forest habitats to result in an overall uniform geographic distribution. In addition, we used 279 locations (some overlap with the locations of the flagging sites, in these occasions we used the more recent data) of proven occurrence records of *I. ricinus* compiled from published sources (see Mihalca et al. 2012b for the list of references consulted). All data were introduced in a database, and we used locality, county and GPS coordinates of each occurrence.

### Environmental variables

To fit the model, we used three different sets of variables. First, the

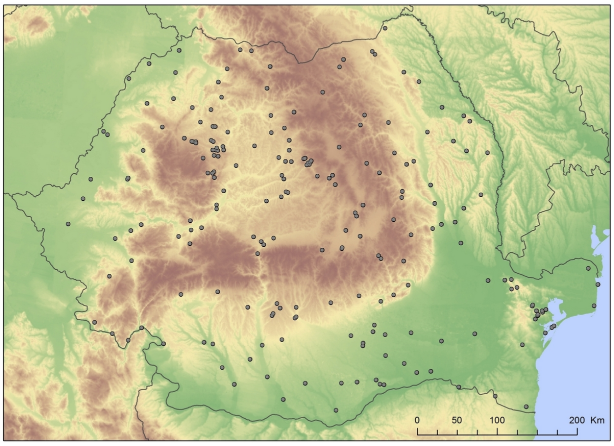


Figure 1. Distribution of cells with individual presence locations of *Ixodes ricinus* used in the distribution model.

climatic data set was provided by WorldClim database (www.worldclim.org). These are high resolution interpolated climate data provided as rasters (Hijmans et al. 2005). To model the probable distribution of the target species we used the bioclimatic dataset. All data were downloaded from WorldClim database with the highest resolution provided; of 30 arc-seconds (one pixel equals ca. 0.6 km²). Nineteen bioclimatic variables provided by the database (derived from monthly temperature, rainfall values and seasonal variations) were downloaded and processed. Although the aim of the study was not to assess the contribution of each variable to the model, we chose to avoid the use of highly correlated variables, and we have eliminated one of the variables in each pair (having a Spearman correlation coefficient $r_s>0.75$). The final set contained fifteen of these climatic variables, and these were used for all models (Table 1.). The second set included only one variable, altitude, which was also provided by the WorldClim database, at a resolution of 30 arc-seconds. The third set also included only one variable, the forest coverage per pixel. The total forested area in each pixel was calculated, and the variable ranges from 0 (no forest) to 1 (100% forest coverage). For the purpose of this analysis, we used the Class 3.1 "Forest" variables, with the following categories: 311 (Broad-leaved forest), 312 (Coniferous forest) and 313 (Mixed forest). The source for this dataset was Corine LandCover database, 2006 version. The dataset was provided by European Environment Agency (EEA, http://www.eea.europa.eu/).

Fitting the model

The model was fitted using presence data for the target species and the environmental variables, using a maximum entropy algorithm, as implemented in the MaxEnt software (version MaxEnt 3.3.1), which has been found to perform better than many different modeling methods (Phillips et al. 2006, Porretta et al. 2013). This approach uses presence-only records and (using the environmental variables) estimates the potential distribution of the species, resulting in a probability of distribution (Phillips et al. 2006).

The model was built using the 75% threshold of occurrence records to construct the model (training data) and the remaining 25% to test it (testing data) (Nenzén & Araújo 2011). The model output was logistic, which provided an estimated relative probability of presence for any given location ranging from 0 (very low probability of species presence) up to 1 (very high relative probability of species presence) (Phillips et al. 2006, Signorini et al. 2014). The rest of the parameters of MaxEnt were set to default. To evaluate the accuracy of the model, we used the area under the curve (AUC) of the receiver operating characteristic (ROC) provided by MaxEnt (Porretta et al. 2013).

To create a numerical characteristic of different geographical zones, we calculated for each county of Romania the number of pixels (as provided by the model output) fulfilling two different criteria.

Table 1. Variables used for building the model and the contribution of each variable.

| Variable | Percent contribution of the variables |
|---|---|
| BIO1 = Annual mean temperature | 2.1 |
| BIO3 = Isothermality (BIO1/BIO7) * 100 | 0.9 |
| BIO6 = Min Temperature of Coldest Period | 0.3 |
| BIO8 = Mean Temperature of Wettest Quarter | 2.0 |
| BIO9 = Mean Temperature of Driest Quarter | 1.2 |
| BIO10 = Mean Temperature of Warmest Quarter | 2.3 |
| BIO11 = Mean Temperature of Coldest Quarter | 0.2 |
| BIO12 = Annual Precipitation | 16.2 |
| BIO13 = Precipitation of Wettest Period | 2.0 |
| BIO14 = Precipitation of Driest Period | 4.3 |
| BIO15 = Precipitation Seasonality | 3.2 |
| BIO16 = Precipitation of Wettest Quarter | 0.0 |
| BIO17 = Precipitation of Driest Quarter | 7.2 |
| BIO18 = Precipitation of Warmest Quarter | 1.2 |
| BIO19 = Precipitation of Coldest Quarter | 0.1 |
| Altitude | 7.1 |
| Forest cover | 49.7 |

The first criterion was the pixel value to be higher than the minimum training presence threshold provided by the model. This criterion represents the climatically suitable area calculated by the model: if higher than the minimum training presence threshold, it means there is a probability that the species may be present. Being a minimum requirement, it does not discriminate between high probability and low probability occurrence areas. In order to achieve this, we used a second criterion, an arbitrary threshold value of 0.5. In this case, we account for the number of pixels per county with a calculated presence probability higher than 50%. In this way, we can discriminate between low probability presence areas and high probability presence areas, thus giving us a supplementary tool in evaluating risk.

As a reference, to calculate the climatically suitable distribution area, the minimum training presence threshold was used and the analysis was run using QGIS (QGIS 2.16) and SAGA (SAGA 2.1.2) software. The raster data were processed and analyzed using SAGA GIS and QGIS software. The models were fitted using MaxEnt software. The final distribution maps were constructed using ArcGIS (ArcGIS 10.3).

## Results

In the case of our model, the value for AUC was 0.724 (for test data), indicating a good performance for our model (Fig. 2). As a reference, to calculate the climatically suitable area, the minimum training presence threshold provided by the model was 0.076.

Analyzing Table 2, and using the 0.5 threshold, we can identify the counties with the highest probability of species' occurrence. Five counties (Alba, Cluj, Mureş, Sibiu and Sălaj, see Fig. 3) have more than 30% of pixels with higher values than 0.5; thus, these areas have highest percent of suitable areas for the species and likely to hold the highest densities of *I. ricinus*.

MaxEnt models also provide a tool to test the variable importance/contribution using the jackknife approach (testing the model with and without a specific variable). In our case, the environmental variable with highest gain is forest cover, which therefore appears to have the most useful information by itself. The environmental variable that decreases gain the most when omitted is again forest coverage, which therefore appears to have the most information that is

Table 2. Distribution of Minimum Threshold Pixel (MTP) values and regions with high risk of presence of *Ixodes ricinus* in individual counties of Romania.

| County | Area (Ha) | Forest coverage (%) | Area covered by pixels with values higher than MTP* (%) | Area covered by pixels with high risk (%) |
|---|---|---|---|---|
| Alba | 624148 | 38.62 | 78.5 | 31.5 |
| Arad | 774793 | 31.43 | 91.1 | 21.1 |
| Argeș | 682269 | 42.61 | 67.0 | 3.1 |
| Bacău | 661807 | 42.46 | 94.7 | 19.9 |
| Bihor | 754997 | 31.41 | 79.6 | 7.6 |
| Bistrița-Năsăud | 535263 | 40.53 | 79.8 | 23.1 |
| Brăila | 476717 | 4.36 | 56.8 | 8.3 |
| Botoșani | 498654 | 10.90 | 66.6 | 8.9 |
| Brașov | 536155 | 41.37 | 76.2 | 28.9 |
| București | 24133 | 2.53 | 30.1 | 4.1 |
| Buzău | 610091 | 26.84 | 88.0 | 5.2 |
| Caraș-Severin | 852099 | 55.73 | 49.1 | 3.1 |
| Călărași | 508707 | 4.09 | 82.4 | 6.0 |
| Cluj | 667151 | 29.78 | 85.6 | 37.8 |
| Constanța | 707184 | 3.52 | 93.2 | 14.4 |
| Covasna | 370718 | 47.22 | 84.6 | 28.1 |
| Dâmbovița | 405306 | 29.78 | 85.9 | 10.9 |
| Dolj | 740991 | 10.47 | 36.4 | 6.0 |
| Galați | 446629 | 7.99 | 90.9 | 12.4 |
| Giurgiu | 354485 | 10.58 | 74.3 | 16.7 |
| Gorj | 558320 | 51.41 | 75.0 | 11.9 |
| Harghita | 664719 | 40.54 | 75.1 | 25.0 |
| Hunedoara | 706152 | 53.65 | 61.7 | 21.5 |
| Ialomița | 445531 | 5.40 | 86.0 | 10.2 |
| Iași | 547705 | 17.58 | 97.3 | 17.6 |
| Ilfov | 156373 | 16.35 | 89.5 | 26.6 |
| Maramureș | 630459 | 48.35 | 52.7 | 0.2 |
| Mehedinți | 495198 | 30.89 | 71.0 | 9.0 |
| Mureș | 671086 | 33.01 | 91.7 | 37.0 |
| Neamț | 588948 | 42.72 | 89.6 | 18.3 |
| Olt | 550531 | 8.82 | 66.2 | 3.7 |
| Prahova | 471417 | 33.40 | 73.6 | 8.4 |
| Satu Mare | 441952 | 17.69 | 71.8 | 7.1 |
| Sălaj | 386411 | 31.08 | 99.3 | 35.3 |
| Sibiu | 542993 | 39.33 | 83.2 | 42.2 |
| Suceava | 855208 | 47.11 | 51.6 | 3.4 |
| Teleorman | 578931 | 4.46 | 56.0 | 4.8 |
| Timișoara | 870417 | 13.77 | 91.3 | 9.4 |
| Tulcea | 849855 | 11.85 | 87.1 | 21.5 |
| Vaslui | 531862 | 13.86 | 88.0 | 9.9 |
| Vâlcea | 576368 | 52.05 | 73.4 | 7.2 |
| Vrancea | 485560 | 40.14 | 84.3 | 14.4 |

not contained in the other variables (Fig. 4).

## Discussion

The geographical distribution, abundance and population structure of ticks in genus *Ixodes* in general, and *I. ricinus* in particular, are related to various and complex factors, including climate and topography (Daniel et al. 2015), the presence of forested habitats and the density of proper hosts (Swanson et al. 2006). For questing ticks, local humidity (determined also by vegetation height) is also an important factor. The distribution and density of questing *I. ricinus* thus is limited by sward height and other physical properties of the vegetation (Mejlon & Jaenson 1997).

*I. ricinus* typically occurs in wooded habitats, primarily in deciduous and mixed forests and their ecotone regions. In areas with high rainfall or elevated local humidity, it may occur in high densities in coniferous forests and in open areas such as grasslands (Gray et al. 1998). Its life cycle is between 2 and 6 years (average 3); however, the duration of the life cycle can vary from one habitat to another and also regionally and is determined by microclimate and host density (Gray 1991).

The aim of this paper was to evaluate the distribution and density of *I. ricinus* throughout Romania, based on environmental predictors, using a set of presence-only records. By building the model, we characterized the whole territory of Romania from the point of view of likeliness of *I. ricinus* presence, prepared a hierarchical list of counties based on distribution-chance, and projected density of this tick vector, using climatic and vegetation conditions of the different counties. The map in Fig. 1 shows a wide modeled distribution for the species in Romania, which is in line with previous studies (Coipan 2010, Mihalca et al. 2012a,b).

Our model showed that the most important predictor of

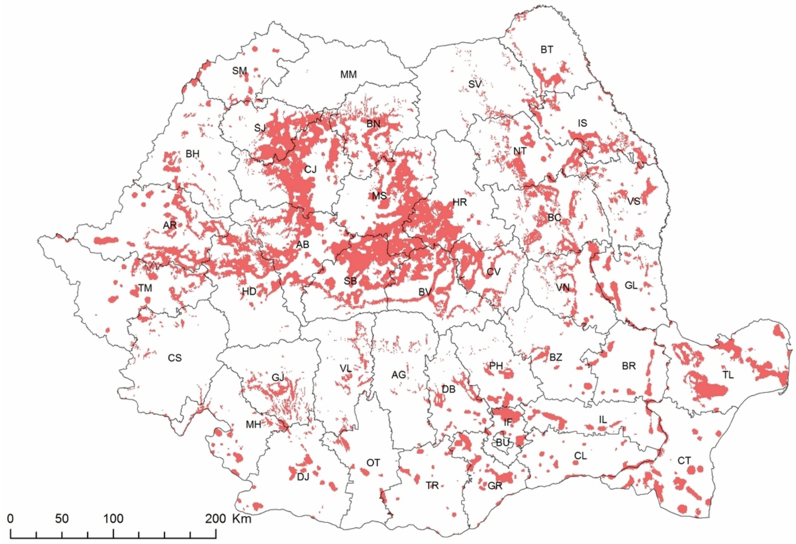


Figure 3. Map of Romania showing the distribution of high-risk areas (red areas are pixels with risk >0.5) for various counties.

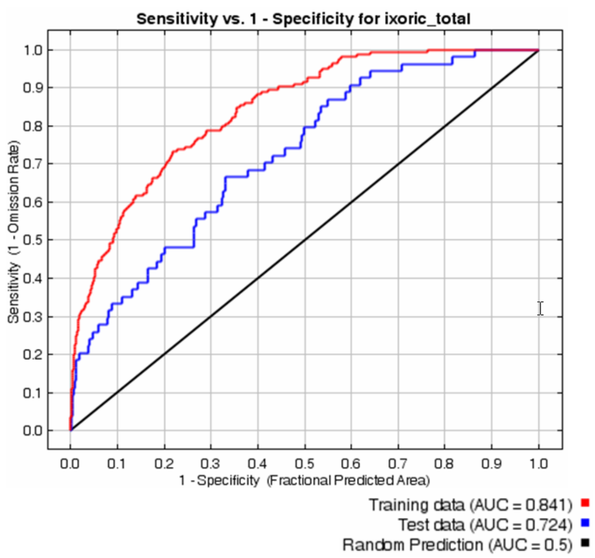


Figure 2. Fitting the model: the receiver operating characteristic (ROC) curve for the dataset.

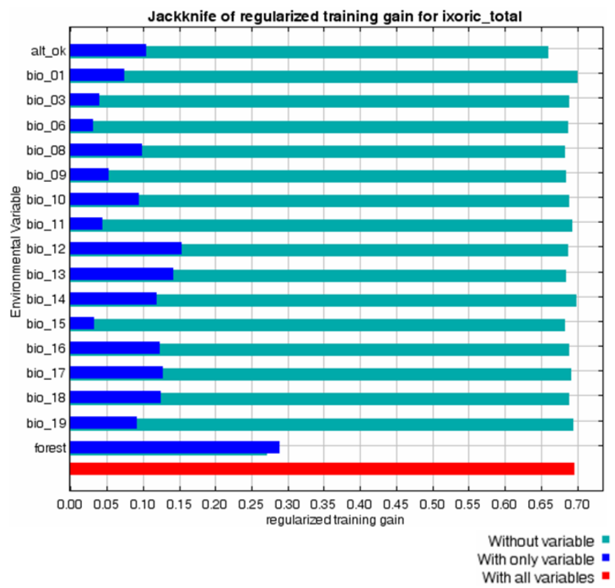


Figure 4. The contribution of each variable using the jackknife technique for testing individual variable importance.

*I. ricinus* presence in a Romania is forest cover (Fig. 2, 4). Although the contribution of any individual variable must be interpreted with caution (Phillips et al. 2006, Porretta et al. 2013), analyzing the result we can see that forest coverage has the largest contribution (49.7%) to our model, followed by annual precipitation amount (16.2 %). Forest cover generally is important for the distribution of *I. ricinus* (Gray et al. 1998, Lindström & Jaenson 2003, Jaenson et al. 2012) and in most modeled distributions may be among the first predictors determining tick distribution and even densities (Daniel et al. 1998, Rizzoli et al. 2009, Jaenson et al. 2012), although local abiotic factors (Daniel et al. 2015) and host densities may also be important (Rizzoli et al 2009). In our case the most important abiotic factor was the annual precipitation amount, which is in line with predictions for the Central European populations of *I. ricinus*, according to Estrada-Pena et al. (2006), thus significantly differing from populations from SE, SW, W or N Europe (see details in Estrada-Peña et al. 2006 for population classifications). Similar results were found by Daniel et al. (2015), who proved that density of questing Central European *I. ricinus* ticks in given areas is highly correlated with monthly rainfall if distribution is corrected for habitat. Host densities are also important for *I. ricinus* presence, however at different spatial scales. Small mammals are important at wider scale by sustaining local tick populations (Lane et al. 1991, Mihalca & Sándor 2013, Martello et al. 2014), while cervids and medium-sized carnivores may influence densities and distribution at local scale, by contributing to tick dissemination and increasing local densities (Lane et al. 1991, Rizzoli et al. 2009). Unfortunately we were not able to control for the effect of host density due to lack of available data.

These results are significant not only from theoretical species distribution, but from epidemiological point of view, too. Preparation of risk maps is important for decision making and has considerable history, with local or global ranges (Bhatt et al. 2013, Kaplan et al. 2014). By mapping the distribution strongholds of *I. ricinus*, one may highlight the geographical risk associated with the presence of this important vector. Surveys of pathogens transmitted by *I. ricinus* in Romania found that pathogen occurrence and density in ticks has a heterogeneous distribution in the country. The highest incidences of TBEV (Coipan et al. 2008), and Lyme-borreliosis (Coipan 2010) in Romania were located in the counties Sibiu, Alba and Cluj, and thus overlapping with our modeled results of likely presence of *I. ricinus* (Fig. 3). Moreover, the genetic diversity of *Borrelia burgddorferi* s.l. genospecies is highest in these counties from all over Romania (Kalmár et al. 2013), in line with previous studies proving that high densities of vectors are associated with high diversity of vectored pathogens (Salkeld et al. 2013).

In conclusion, modeling the distribution of parasites using climate and topography may provide distribution maps that can predict the risk associated with high incidence of parasite distribution, and as such they may offer a new tool for targeted epidemiologic studies or the development of locally adapted tick-borne disease prevention policies.

**Acknowledgements.** The research was funded by POSDRU grant no. 159/1.5/S/136893 grant with title: "Parteneriat strategic pentru creșterea calității cercetării științifice din universitățile medicale prin acordarea de burse doctorale și postdoctorale – DocMed.Net_2.0" for CD, the work of ADS was funded by PN-II- RU-TE- 2014-4- 1389, while ADM was beneficiary of PN-II- RU-TE- 2014-4- 091 grant. This paper was published under the frame of EurNegVec COST Action TD1303..

**References**

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Myers, M.F. (2013): The global distribution and burden of dengue. Nature 496(7446): 504-507.

Briciu, V.T., Titilincu, A., Țățulescu, D.F., Cârstina, D., Lefkaditis, M., Mihalca, A.D. (2011): First survey on hard ticks (Ixodidae) collected from humans in Romania: possible risks for tick-borne diseases. Experimental and Applied Acarology 54: 199-204.

Coipan, E.C. (2010): Hard ticks (Acari: Ixodidae) – vectors for Lyme disease spirochetes in Romania. Romanian Journal of Biology, Zoology 55: 177-184.

Coipan, E.C., Alexse, A., Vladimirescu, A.F., Nicolescu, G. (2008): Seropositivity among human subjects for both TBEV and *Borrelia burgdorferi* sl. during a TBE outbreak in Sibiu County, Romania. International Journal of Infectious Diseases 12: e334.

Coipan, E.C., Jahfari, S., Fonville, M., Maassen, C.B., van der Giessen, J., Takken, W., Takumi, K., Sprong, H. (2013): Spatiotemporal dynamics of emerging pathogens in questing *Ixodes ricinus*. Frontiers in Cellular and Infection Microbiology 3: 36.

Daniel, M., Kolár, J., Zeman, P., Pavelka, K., Sádlo, J. (1998): Predictive map of *Ixodes ricinus* high-incidence habitats and a tick-borne encephalitis risk assessment using satellite data. Experimental and Applied Acarology 22: 417-433.

Daniel, M., Malý, M., Danielová, V., Kříž, B., Nuttall, P. (2015): Abiotic predictors and annual seasonal dynamics of *Ixodes ricinus*, the major disease vector of Central Europe. Parasites & Vectors 8: 1.

van Duijvendijk, G., Sprong, H., Takken, W. (2015): Multi-trophic interactions driving the transmission cycle of *Borrelia afzelii* between *Ixodes ricinus* and rodents: a review. Parasites & Vectors 8: 4.

Estrada-Peña, A., Venzal, J.M., Sánchez Acedo, C. (2006): The tick *Ixodes ricinus*: distribution and climate preferences in the western Palaearctic. Medical and Veterinary Entomology 20: 189-197.

Gray, J.S. (1991) The development and seasonal activity of the tick *Ixodes ricinus*: a vector of Lyme borreliosis. Revue of Medical and Veterinary Entomology 79: 323-333.

Gray, J.S., Kahl, O., Robertson, J.N., Daniel, M., Estrada-Pena, A., Gettinby, G., Jaenson, T.G.T., Jaenson, P., Jongejan, F., Korenberg, E., Kurtenbach, K., Zeman, P. (1998): Lyme borreliosis habitat assessment. Zentralblatt für Bakteriologie und Hygiene 287: 211-228.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A. (2005): Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965-1978.

Jaenson, T.G., Jaenson, D.G., Eisen, L., Petersson, E., Lindgren, E. (2012): Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. Parasites & Vectors 5: 8.

Jore, S., Vanwambeke, S.O., Viljugrein, H., Isaksen, K., Kristoffersen, A.B., Woldehiwet, Z., Johansen, B., Brun, E., Brun-Hansen, H., Wstermann, S., Larsen I.L., Ytrehus, B., Hofshagen, M. (2014): Climate and environmental change drives *Ixodes ricinus* geographical expansion at the northern range margin. Parasites & Vectors 7: 11.

Kalmár, Z., Mihalca, A.D., Dumitrache, M.O., Gherman, C.M., Magdaş, C., Mircean, V., Oltean, M., Domşa, C., Matei, I.A., Mărcuțan, D.I., Sándor, A.D., D'Amico, G., Paştiu, A., Györke, A., Gavrea, R., Marosi, B., Ionică, A., Burkhardt, E., Toriay, H., Cozma, V. (2013): Geographical distribution and prevalence of *Borrelia burgdorferi* genospecies in questing *Ixodes ricinus* from Romania: a countrywide study. Ticks and Tick-borne Diseases 4(5): 403-408.

Kaplan, H., van Niekerk, A., Le Roux, J.J., Richardson, D.M., & Wilson, J.R. (2014): Incorporating risk mapping at multiple spatial scales into eradication management plans. Biological Invasions 16: 691-703.

Lane, R.S., Piesman, J., Burgdorfer, W. (1991): Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annual Review of Entomology 36: 587-609.

Lindström, A., Jaenson, T.G. (2003): Distribution of the common tick, *Ixodes ricinus* (Acari: Ixodidae), in different vegetation types in southern Sweden. Journal of Medical Entomology 40: 375-378.

Martello, E., Mannelli, A., Ragagli, C., Ambrogi, C., Selmi, M., Ceballos, L.A., Tomassone, L. (2014): Range expansion of *Ixodes ricinus* to higher altitude, and co-infestation of small rodents with *Dermacentor marginatus* in the Northern Apennines, Italy. Ticks and Tick-borne Diseases 5(6): 970-974.

Materna, J., Daniel, M., Danielová, V. (2005): Altitudinal distribution limit of the tick *Ixodes ricinus* shifted considerably towards higher altitudes in central Europe: results of three years monitoring in the Krkonose Mts. (Czech Republic). Central European Journal of Public Health 13: 24-28.

Mejlon, A., Jaenson, T.G.T. (1997): Questing behaviour of *Ixodes ricinus* ticks (Acari: Ixodidae). Experimental and Applied Acarology 21:747-754.

Mihalca, A.D., Gherman, C.M., Magdaș, C., Dumitrache, M.O., Györke, A., Sándor, A.D., Domșa, C., Mircean, V., Mărcuțan, D.I., D'Amico, G., Păduraru, A.O., Cozma, V. (2012a): *Ixodes ricinus* is the dominant questing tick in forest habitats from Romania: the results from a countrywide dragging campaign. Experimental and Applied Acarology 58: 175-182.

Mihalca, A.D., Dumitrache, M.O., Magdaş, C., Gherman, C.M., Domşa, C., Mircean, V., Ghira, I.V., Pocora, V., Cozma, V., Sándor, A.D. (2012b): Synopsis of the hard ticks (Acari: Ixodidae) of Romania with update on host associations and geographical distribution. Experimental and Applied Acarology 58: 183-206.

Mihalca, A.D., Sándor, A.D. (2013): The role of rodents in the ecology of *Ixodes ricinus* and associated pathogens in Central and Eastern Europe. Frontiers in Cellular and Infection Microbiology 3: 56.

Nenzén, H.K., Araújo, M.B. (2011): Choice of threshold alters projections of species range shifts under climate change. Ecological Modeling 222: 3346-3354.

Ostfeld, R.S., Brunner, J.L. (2015): Climate change and *Ixodes* tick-borne diseases of humans. Philosophical Transactions of the Royal Society London B. Biological Sciences 370: 20140051.

Phillips, S.J., Anderson, R.P., Schapire, R.E. (2006): Maximum entropy modeling of species geographic distributions. Ecological Modeling 190: 231-259.

Porretta, D., Mastrantonio, V., Amendolia, S., Gaiarsa, S., Epis, S., Genchi, C., Bandi, C., Otranto, D., Urbanelli, S. (2013): Effects of global changes on the climatic niche of the tick *Ixodes ricinus* inferred by species distribution modelling. Parasites & Vectors 6: 271.

Rizzoli, A., Hauffe, H.C., Tagliapietra, V., Neteler, M., Rosà, R. (2009): Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy. Plos One 4: e4336.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Spitalská, E., Kazimírová, M. (2014): *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. Frontiers in Public Health 2: 251.

Salkeld, D.J., Padgett, K.A., Jones, J.H. (2013): A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters 16: 679-686.

Sándor, A.D., D'Amico, G., Gherman, C.M., Dumitrache, M.O., Domșa, C., Mihalca, A.D. (2017): Mesocarnivores and macroparasites: altitude and land-use predict the ticks occurring on red foxes (*Vulpes vulpes*). Parasites & Vectors 10: 173.

Signorini, M., Cassini, R., Drigo, M., Frangipane di Regalbono, A., Pietrobelli, M., Montarsi, F., Stensgaard, A.S. (2014): Ecological niche model of *Phlebotomus perniciosus*, the main vector of canine leishmaniasis in north-eastern Italy. Geospatial Health 9: 193-201.

Swanson, S.J., Neitzel, D., Reed, K.D., Belongia, E.A. (2006): Coinfections acquired from *Ixodes* ticks. Clinical Microbiology Review 19: 708-727.