Ecological niche modelling of *Babesia* spp. infection in wildlife experimentally evaluated in northern Italy with reference to questing *Ixodes ricinus* ticks

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Abstract

Tick-borne diseases and especially those caused by protozoa of the genus *Babesia*, are gaining increasing attention as emerging zoonotic pathogens. Zoonotic species like *B. venatorum* and *B. microti* have wild animals as main reservoir hosts. We propose a habitat suitability model for *Babesia* spp. to better understand the entity of *Babesia* presence, to improve diagnostic awareness and to optimize screening and preventive actions. The probability of *Babesia* spp. presence was estimated by investigation of potential correlation between this protozoa in wild ruminants and the environmental factors that can favour or limit vector and host availability. We developed two separate models to evaluate the separate roles of cervids and alpine chamois for *Babesia* spp. epidemiology. A comprehensive model using all presence data from all ungulates species was also developed. The overall area suitable for *Babesia* spp. in this simulation was found to be 3,723 km^2, which corresponds to 15.51% of the background regional territory. The model developed was empirically validated assessing tick abundance in randomly chosen areas classified by the model as moderately or highly suitable for *Babesia* spp. Collected Ixodidae ticks analysed for *Babesia* infection by molecular analysis, to confirm the model predictions, indicated a significantly higher prevalence of this infection in areas identified by the model as highly suitable compared to areas classified as only moderately so (X^2= 5.05, p<0.05, Odds Ratio= 2.12, Confidence Interval 95%= 1.1-4.1).

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Introduction

Piroplasmosis, caused by protozoa of the genus *Babesia*, is gaining increasing attention globally as an emerging zoonosis (Hong *et al.*, 2019; Svensson *et al.*, 2019). *Babesia* is the most frequent parasite transmitted by blood transfusion in North America (Vannier and Krause, 2012; Ryan *et al.*, 2020) and it causes high morbidity and mortality in livestock in endemic tropical and subtropical areas (Kivaria *et al.*, 2007; Pfeffer *et al.*, 2018). Despite the economic and public health importance, little information is available regarding the parasite-host-vector-environment interface (Pérez De León *et al.*, 2010). The epidemiology of a disease may be complex, especially if a vector is required (*e.g.*, Ixodidae ticks for *Babesia* spp.) and if many species can be reservoirs or dead-end hosts. To better understand the epidemiology of circulating tick-borne piroplasms, we modelled *Babesia* spp. occurrence in wild ruminants that are among the main hosts of *Ixodes ricinus* and also play the role of a viable platform for systemic and non-systemic pathogens transmission among co-feeding ticks (Kiffner *et al.*, 2010). We targeted three host species: roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and alpine chamois *(Rupicapra rupicapra*) for which prevalence data of *Babesia* spp. infection, within the study area, were available (Zanet *et al.*, 2014). *C. capreolus* is the host species with the highest rates of infection, ranging from 12.6% to 83.6% (Bonnet *et al.*, 2007; Tampieri *et al.*, 2008; Malandrin *et al.*, 2010; Zanet *et al.*, 2014; Razanske *et al.*, 2019). It is the reservoir host of *B. capreoli* (Yabsley and Shock, 2013) and of the zoonotic *B. venatoroum* (Malandrin *et al.*, 2010). Red deer and chamois are highly susceptible to *Babesia* infection (Yabsley and Shock, 2013). In alpine chamois frequent fatal cases have been reported (Hoby *et al.*, 2007, 2009) as consequences of spillover from sympatric roe deer (Hoby *et al.*, 2007, 2009; Tampieri *et al.*, 2008). In north-western Italy (the Piedmont region) the roe deer population is experiencing a steady increase and territorial expansion (Carnevali *et al.*, 2009) that have driven a concomitant increase of ticks, especially *I. ricinus* which is the most common tick of temperate regions (Vor *et al.*, 2010; Rizzoli *et al.*, 2014).

The modelling effort of the present study was focused on the environmental requirements of this tick species (Estrada-Peña, 2001a). Species Distribution Models (SDM) are produced by relating species occurrence data (*i.e*. *Babesia* infection) to a set of environmental variables reflecting key factors for species occurrence, such as climate, topography, geology or land-cover. Ecological Niche Models (ENMs) describe the niche of a species by describing its most suitable habitat (Pulliam, 2000; Sillero, 2011). We developed separate models for cervids (roe deer and red deer) and chamois to account for the different roles of reservoir and spillover hosts, respectively. The proposed ENM for *Babesia* spp. can serve as tool in human and veterinary health to understand the distribution of *Babesia* spp. presence, to improve diagnosis of cryptic clinical cases in humans and to facilitate the efficacy of monitoring, control and preventive efforts. The developed ENM was experimentally evaluated by assessing tick abundance in the environment and by analysing ticks collected in areas identified by the model as moderately or highly suitable for *Babesia spp*.

Materials and Methods

Study site

Species distribution modelling

Factors affecting the distribution of *Babesia* spp. were estimated using MaxEnt 3.3.3 (Phillips *et al.*, 2006). The algorithm implemented in MaxEnt estimates a target probability distribution (*i.e*. *Babesia* spp.) by finding the probability distribution of maximum entropy (*i.e*. most spread out or closest to uniform) within a set of constraints that represent the available information about the environmental requirements of our target distribution (Phillips *et al.*, 2006). We adhered to the default settings for the regularization multiplier, maximum number of iterations, convergence threshold and maximum number of background points that were limited to the Piedmont administrative division with exclusion of all urban areas as unsuitable for piroplasms hosts and vectors (Hirzel *et al.*, 2006; Elith *et al.*, 2011). We generated models randomly assigning 75% of occurrences as training data with the remaining 25% used as test data. We ran five cross-validate replicates for each model. Selection of 'features' (predictors) was carried out automatically following the default rules dependent on the number of presence records (Phillips *et al.*, 2006; Elith *et al.*, 2011). We used the logistic output format conditioned on the environmental variables in each grid cell with suitability values ranging from 0 (unsuitable environmental condition) to 1 (optimal environmental condition) (Hu and Jiang, 2011). The threshold value above which there is a substantial probability of presence of *Babesia* spp. was set for each model to the value that maximizes training sensitivity and specificity (Phillips *et al.*, 2006; Elith *et al.*, 2011). Values of habitat suitability above the threshold were divided in four quartiles for graphical visualization and further on-field validation. Model evaluation and best-model selection were carried out using the Area Under the receiver-operator Curve (AUC) (Merow *et al.*, 2013) and lower model complexity (Elith and Leathwick, 2009). The best performing model ($\Delta AUC \leq 2$) and lower number of covariates was chosen. Covariates to be retained were selected by backward stepwise model selection excluding the feature with the lowest permutation importance at each step (Merow *et al.*, 2013).

Infection: presence only data

A total of 61 animals, positive by the polymerase chain reaction (PCR) for *Babesia* spp., were used as Presence Points (PP) to train the MaxEnt model. Specifically, roe deer (n=30), red deer (n=23) and alpine chamois (n=8) are the three ungulate species included in the model. Positivity to *Babesia* spp. in these animals has been assessed previously (Zanet *et al.*, 2014). The PP were georeferenced by Global Positioning System (GPS) coordinates (UTM, datum ED50, Fuse 32N) which refer to the location where the animals were killed by hunting or found dead. We developed three models to estimate the probability of presence of *Babesia* spp. in Piedmont. The first one considered only cervids (Model 1) as target hosts, and we used PP data of *Babesia* spp. isolated from either red deer or roe deer. Presence of red deer and roe deer habitat can overlap, whereby tick-borne diseases are shared among both species (Duh *et al.*, 2005; Tampieri *et al.*, 2008; Bastian *et al.*, 2012). Cervids are the primary source of blood meals for nymphs and adult *I. ricinus* (Cadenas *et al.*, 2007; Humair *et al.*, 2007) and their abundance is directly related to deer presence and abundance in the area (Wilson *et al.*, 1990). The second model was trained with alpine chamois PP data only, as this species is usually found at higher elevations and in an environment different from where cervids are typically found, even if a partial overlap between the two species can occur (Model 2). For *Babesia* spp, chamois is primarily a dead-end, spillover host. (Hoby *et al.*, 2009). The third model used all PP records from cervids and chamois together to summarize *Babesia* spp. circulation in the three most frequently infected wild ungulate species in Piedmont (Model 3).

Candidate predictors

A total of 21 predictors were selected based on the ecological requirements of *Babesia* definitive hosts and vectors (Table 1).

Table 1. Candidate modelling predictors.

Host species predictor	Tick vector predictor
Roe deer hunting bag consistency	Altitude
Red deer hunting bag consistency	Land cover
Chamois hunting bag consistency	Annual solar exposure
Ungulate hunting bag consistency	Summer/winter brightness temperature (min, max, mean)
	Summer/winter NDVI (min, max, mean)
	Surface aspect
	Surface slope

A total of 21 host (n=4) and environmental (n=17) factors were evaluated as potential predictors for MaxEnt models.

Biotic and abiotic factors influencing the presence of *I. ricinus* were chosen considering the available information on piroplasmid infections in wild ungulates in Europe (Duh *et al.*, 2005; Hoby *et al.*, 2007, 2009; Tampieri *et al.*, 2008; Malandrin *et al.*, 2010; Zintl *et al.*, 2011; Bastian *et al.*, 2012; Overzier *et al.*, 2013; Rizzoli *et al.*, 2014; Ebani *et al.*, 2016; Cézanne *et al.*, 2017; Kauffmann *et al.*, 2017; Remesar *et al.*, 2019). The spatial resolution of all predictors as well as of that of the final model was 1000 m.

Host species predictors

Presence and hunting bag numerical consistency (roe deer, red deer and chamois) were inferred from the Official Hunting dataset (Osservatorio Faunistico Regione Piemonte).

Tick vector predictors

Five abiotic candidate priors were included in the model: i) altitude (ALT); ii) annual solar exposure (total KWatthour/m^2 received during a year period (SOLAR); iii) surface aspect (slope direction of the maximum rate of change in value from each cell to its neighbours (ASPECT); iv) slope (steepness of land surface measured in degrees from the horizontal (SLOPE) - original resolution 250 m (http://webgis.arpa.piemonte.it); and v) Brightness Temperature (BT) - resolution 1000 m (http://glovis.usgs.gov). Among the biotic priors, the performance of the Normalized Difference Vegetation Index (NDVI) and land cover were tested. NDVI was considered separately for two periods: i) from May to September (vegetation peak season, n=34 Landsat images) and ii) from November to March (minimum vegetation presence, n=29 Landsat images) - resolution 1000 m (http://glovis.usgs.gov). For each season, we evaluated the fit of PP data on the minimum, maximum and mean NDVI that was used as indicator of vegetation coverage and soil relative humidity (Estrada-Peña, 2001a). The same Landsat image collection was used for calculating summer and winter BT (minimum, maximum and mean BT) as indicator of the relative ground temperature (Eisen *et al.*, 2010; Hönig *et al.*, 2011). The land cover raster map (http://www.ruparpiemonte.it/geocatalogorp/main/?sezione=catalogo) was also evaluated as a model covariate (LAND COVER). To exclude autocorrelation among covariates (cor), independence was assessed using a correlation matrix.

Output analysis and visualization

MaxEnt graphical outputs were analyzed and visualized using QuantumGIS 3.4 (QGIS Developmental Team, 2018). The statistical analysis was performed using R 3.4.4 (R Development Core Team, 2018).

Experimental model evaluation of questing ticks

Tick collection and biomolecular analysis

To experimentally confirm the validity of the model, we randomly selected 8 locations uniformly distributed across the study area which were classified by Model 3 as moderately suitable (locations Q2 - 4) and highly so (locations Q4 - 4) for *Babesia* spp. Model 3 was used as reference for empirical validation to include the broadest possible range for *Babesia* spp. circulation within the study area. In each of these areas, we sampled monthly for questing Ixodida*e* ticks by dragging. Forty transects (5 transects for each location, each transect covering an area of 100 m^2) were sampled each month from May to October. Before each sampling session the exact coordinates of the sampled transect, temperature and humidity were recorded. Collected ticks were preserved in 70% ethanol and identified with reference to species, life-stage and gender under a stereomicroscope using appropriate morphological keys (Walker, 2003; Estrada-Peña *et al.*, 2017). Identified ticks were divided into pools comprising specimens collected from the same transect and homogeneous for species, developmental stage and sex. The tick pools were mechanically homogenized using Qiagen TissueLyser LT (Qiagen, Milan, Italy). Total genomic DNA was extracted according to Maurelli *et al.* (2018) and the V4 hypervariable region of the 18S rDNA amplified by a semi-nested PCR methodology (Zanet *et al.*, 2017). All PCR-positive amplicons were purified using a commercial kit (Nucleospin Extract II Kit, Macherey-Nagel, Düren, Germany) and both strands sequenced (Macrogen Europe, Spain) for species identification. The resulting nucleotide sequences were analyzed using MEGA X software (Kumar *et al.*, 2018) and compared to those available in GenBank (http://www.ncbi.nlm.nih.gov/genbank). The PCR results were expressed as a minimum infection rate (MIR) or the minimum percentage of ticks in a pool with detectable DNA of *Babesia* spp. (Kramer *et al.*, 1999).

Statistical analysis

Mean tick abundance in the Q2 and Q4 locations was compared using Student's T test for paired data. To identify variables associated with *Babesia* spp. infection in ticks collected in the environment we calculated using Chi Square test (X^2) and Odd Ratio (OR). Differences were considered significant at $p<0.05$. All analysis was performed using R 3.5.3 (R Development Core Team, 2018).

Results

Assessment of covariate independence

The correlation matrix of covariates dependence allowed us to exclude from contemporary use in MaxEnt analysis SLOPE and ALT (cor=0.71), maximum summer BT and ALT (cor=0.70).

Model 1 - Babesia spp. distribution in cervids

The best performing model (AUC= 0.937, Figure 1) for *Babesia* spp. occurrence in cervids was generated using the following parameters as covariates: roe deer and red deer abundance, mean summer NDVI, SLOPE, SOLAR and LAND COVER (see Figure 2 for the response curves of each feature and for the jackknife test of variable importance on training gain and on AUC). Red deer abundance, together with mean summer NDVI, SLOPE and SOLAR had a direct correlation with *Babesia* spp. occurrence probability. *Babesia* spp. suitability peaked with roe deer abundance of 4 individuals/km^2 and the most suitable land use categories were found to be broad-leaved forest (category 9), ecotonal shrub areas (category 10), high altitude pastures and meadows (category 14) and grasslands (category 7). The suitable area for *Babesia* spp. infection in wild cervids was estimated at 956 km^2 (about 4% of the regional territory; threshold value for Maximum Training Sensitivity plus Specificity 0.443) (Table 2, Figure 3).

Model 2- Babesia spp. distribution in alpine chamois

The best performing model (AUC= 0.993, Figure 1) for *Babesia* spp. in Alpine chamois was generated using the following parameters as covariates: chamois density, ALT, SOLAR LAND

COVER and mean summer NDVI (see Figure 3 for the response curves of each feature, and the jackknife test of variable importance on training gain and on AUC). All continuous variables had a direct correlation with *Babesia* spp. probability, while the land cover categories most suitable to infection were found to be high altitude pastures and meadows (category 14), grasslands (category 7) and ecotonal shrub areas (category 10). The suitable area for *Babesia* spp. infection in chamois was estimated at 81 km^2 (0.35% of the regional territory; threshold value for Maximum Training Sensitivity plus Specificity 0.465) (Table 3).

Table 2. Suitable area for *Babesia* spp. infection in cervids.

Probability of presence	Area (km^2)	Percent of the area of presence	Percent of the area of presence
1st Quartile	206	21.58%	0.86%
2nd Quartile	211	22.09%	0.88%
3rd Quartile	226	23.65%	0.94%
4th Quartile	313	32.68%	1.30%
Total area of presence	956	100.00%	3.98%

The predicted presence area for Babesia spp. infection was divided into quartiles of growing probability of presence. The size of the suitable area (km^2), the percent of the total presence area (above minimum threshold of 0.443) and the percent on the background (Piedmont Region without urbanized areas) is given for each quartile.

Table 3. Suitable area for *Babesia* spp. infection in chamois.

Probability of presence	Area (km^2)	Percent of the area of presence	Percent of the area of presence
1st Quartile	29	35.19%	0.12%
2nd Quartile	18	22.30%	0.08%
3rd Quartile	17	20.37%	0.07%
4th Quartile	17	22.15%	0.08%
Total area of presence	81	100.00%	0.35%

The predicted presence area for Babesia spp. infection was divided into quartiles of growing probability of presence. The size of the suitable area (km^2), the percent of the total presence area (above minimum threshold of 0.443) and the percent on the background (Piedmont Region without urbanized areas) is given for each quartile.

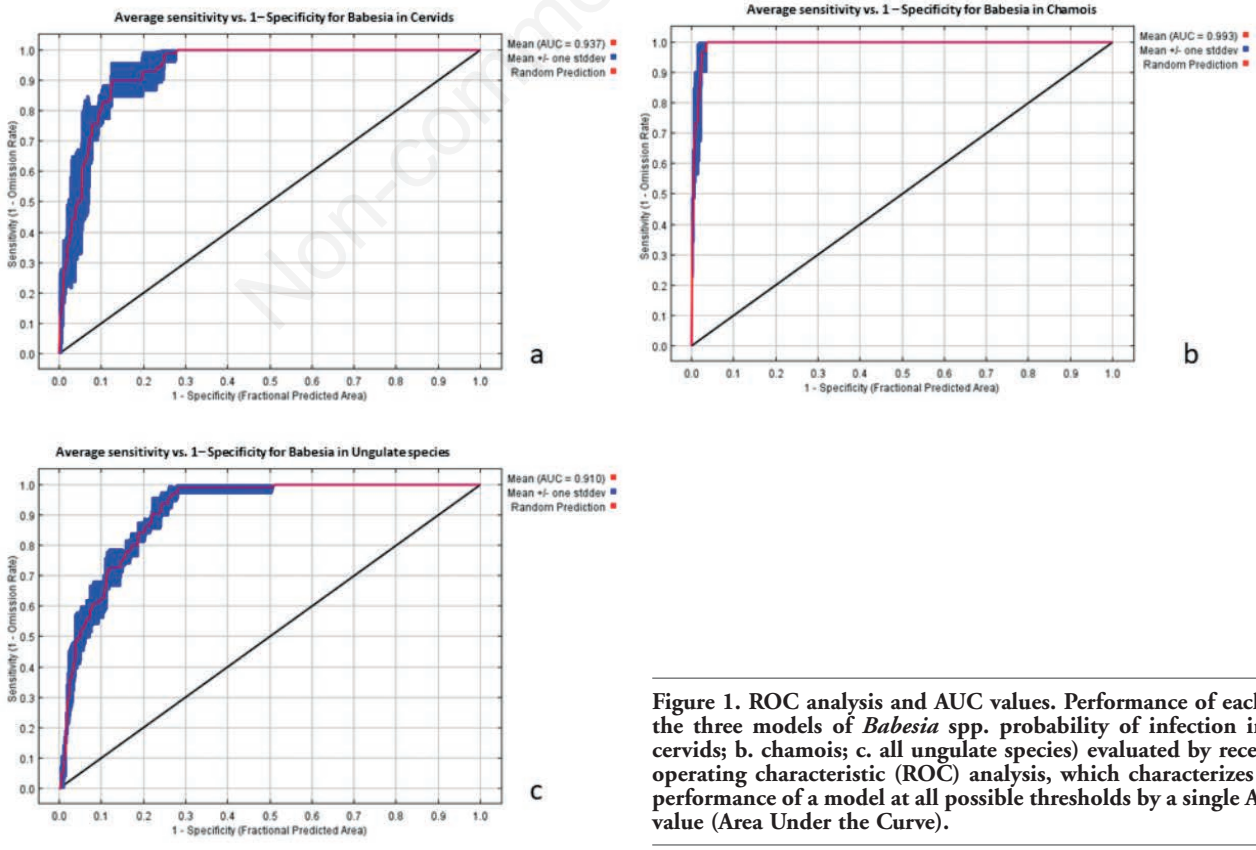

Figure 1. ROC analysis and AUC values. Performance of each of the three models of *Babesia* spp. probability of infection in a. cervids; b. chamois; c. all ungulate species) evaluated by receiver operating characteristic (ROC) analysis, which characterizes the performance of a model at all possible thresholds by a single AUC value (Area Under the Curve).

Model 3 - Overall Babesia spp. distribution in Piedmont

The occurrence of *Babesia* spp. was also estimated for cervids and chamois together, as there is evidence that natural infection in alpine chamois is frequently acquired from sympatric roe deer (Hoby *et al.*, 2007, 2009; Tampieri *et al.*, 2008; Zanet *et al.*, 2014). The overall suitable area for *Babesia* spp. in this simulation was estimated at 3,723 km^{2,} which corresponds to approximately 15.5% of the background regional territory (Figure 4). The AUC of the model was 0.91 (Figure 1). distribution of occurrence probability is reported in Table 4. Retained covariates were: mean summer NDVI, SOLAR, and LAND COVER (Figure 5). The probability of presence for *Babesia* spp., peaked when NDVI reaches 0.65, value that corresponds to broad-leaved forests areas, that are notoriously the most suitable environments for *Ixodidae* ticks(Daniel *et al.*, 1998; Estrada-Peña, 2001b; Estrada-Peña and Venzal, 2006). Land cover confirmed the high suitability of broad-leaved forest areas (class 9, in the LAND COVER predictor) and solar exposure showed a direct correlation with increasing *Babesia* spp. habitat suitability.

Field model validation

Over the 6 months of sampling, we collected 1,555 ticks, which were morphologically identified as *I. ricinus* (n=1,553) and

Table 4. Suitable area for *Babesia* spp. infection in wild ungulates.

Probability of presence	Area (km^2)	Percent of the area of presence	Percent of the area of presence
1st Quartile	883	23.70%	3.68%
2nd Quartile	824	22.13%	3.43%
3rd Quartile	899	24.16%	3.75%
4th Quartile	1,117	30.01%	4.65%
Total area of presence	3,723	100.00%	15.51%

The predicted presence area for Babesia spp. infection was divided into quartiles of growing probability of presence. The size of the suitable area (km^2), the percent of the total presence area (above minimum threshold of 0.443) and the percent on the background (Piedmont Region without urbanized areas) is given for each quartile.

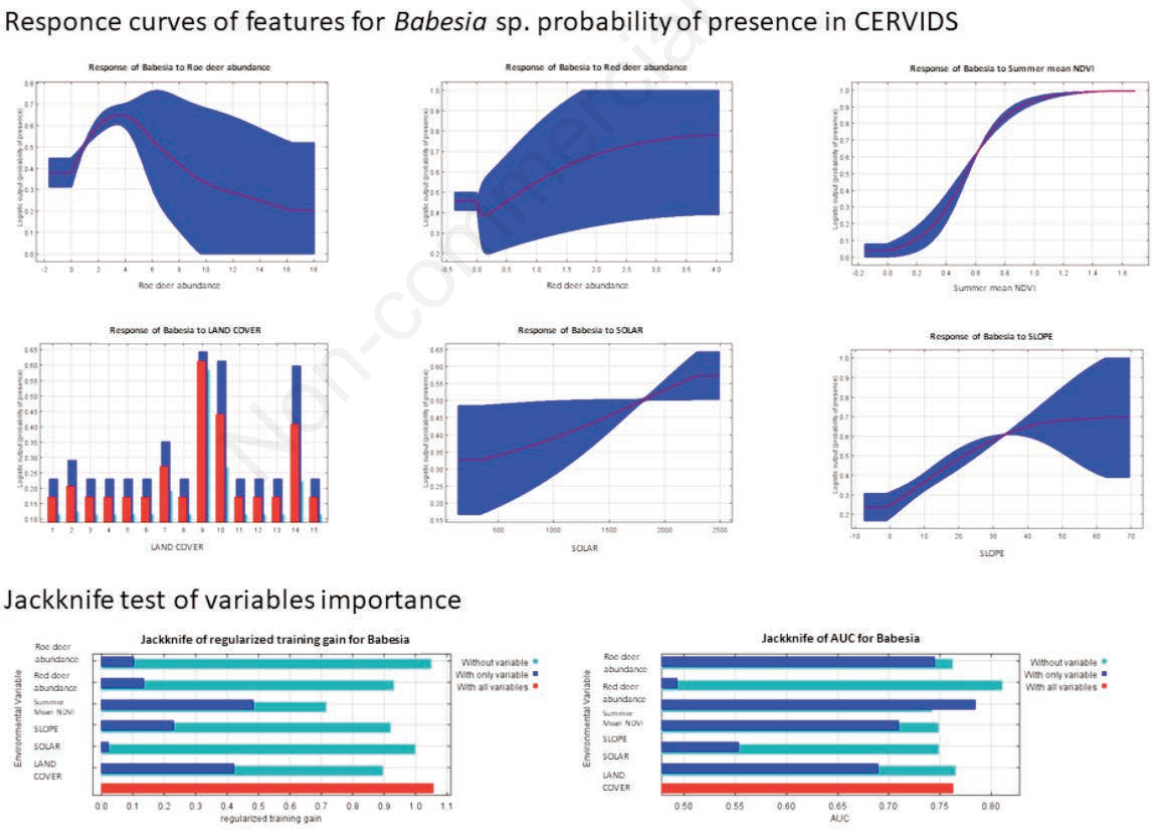

Figure 2. Covariate response curves and Jackknife test of variable importance – Model 1 cervids. Response curves of the selected covariates for *Babesi*a spp. occurrence in cervids. Environmental covariates (mean summer NDVI, solar exposure and slope) and host abundance are directly related to increased *Babesia* spp. suitability. Land cover classes mostly associated with Babesia spp. suitability are pasture (cat.7), broad-leaved forest (cat.9), shrubs (cat. 10), and alpine meadows and pastures (cat.14). Jackknife test of variable importance shows the impact of each variable on training gain and AUC. Values shown are averages over replicate runs.

I. hexagonus (n=2). The number of ticks collected from each location is reported in Table 5. Larvae were the most abundant life-stage in all sampling locations (n=1302), followed by nymphs (n=250) and adults (n=3). Identified ticks were divided, for molecular analysis, in 161 homogeneous pools (pools of adult ticks n=3, nymphs n=74 and larvae n=84). *Babesia* spp. DNA was detected in 59 pools (MIR=36.65%; 95% CI 29.6-44.32). The highest MIR (p<0.05) was recorded in adults (MIR=100.00%; 95% CI 43.85-100) followed by larvae (MIR=36.90%; 95% CI 27.37-47.58) and nymphs (MIR=33.78%; 95% CI 24.05-45.12). From locations moderately suitable for *Babesia* spp. (Q2) we consistently recovered a lower number of ticks compared to highly suitable areas (Q4) with exception of one Q4 location (location F, Table 5) where no ticks were recovered. Results from location F were possibly impaired by the use of part of the sampling area as pasture for cattle. *Babesia* spp. MIR was significantly higher in Q4 areas than in Q2 areas (X^2=5.05 p<0.05) with OR=2.12 (95% CI 1.10-4.10). The zoonotic *B. venatorum* was the most prevalent species with a MIR of 23.60% (95% CI 17.71 – 30.73). *B. capreoli* was reported with an overall MIR of 3.11% (95% CI 1.33 – 7.06) followed by *B. microti* and *B. vulpes,* which were both detected with a MIR of 2.48% (95% CI 0.97 – 6.21%). Protozoa of the genus *Theileria (T. buffeli* group) were detected in 8 pooled samples with a MIR of 4.97% (95% CI 2.54 – 9.50%). All species were detected with a higher prevalence in Q4 areas compared to Q2 (Table 6).

Discussion

The models presented are intended as a tool to better understand the geographical occurrence and epidemiology of *Babesia* piroplasms infecting wild ungulates. Babesiosis plays a key pathogenic role in livestock (Criado-Fornelio *et al.*, 2003; Schnittger *et al.*, 2003; Bock *et al.*, 2008) and as emerging disease

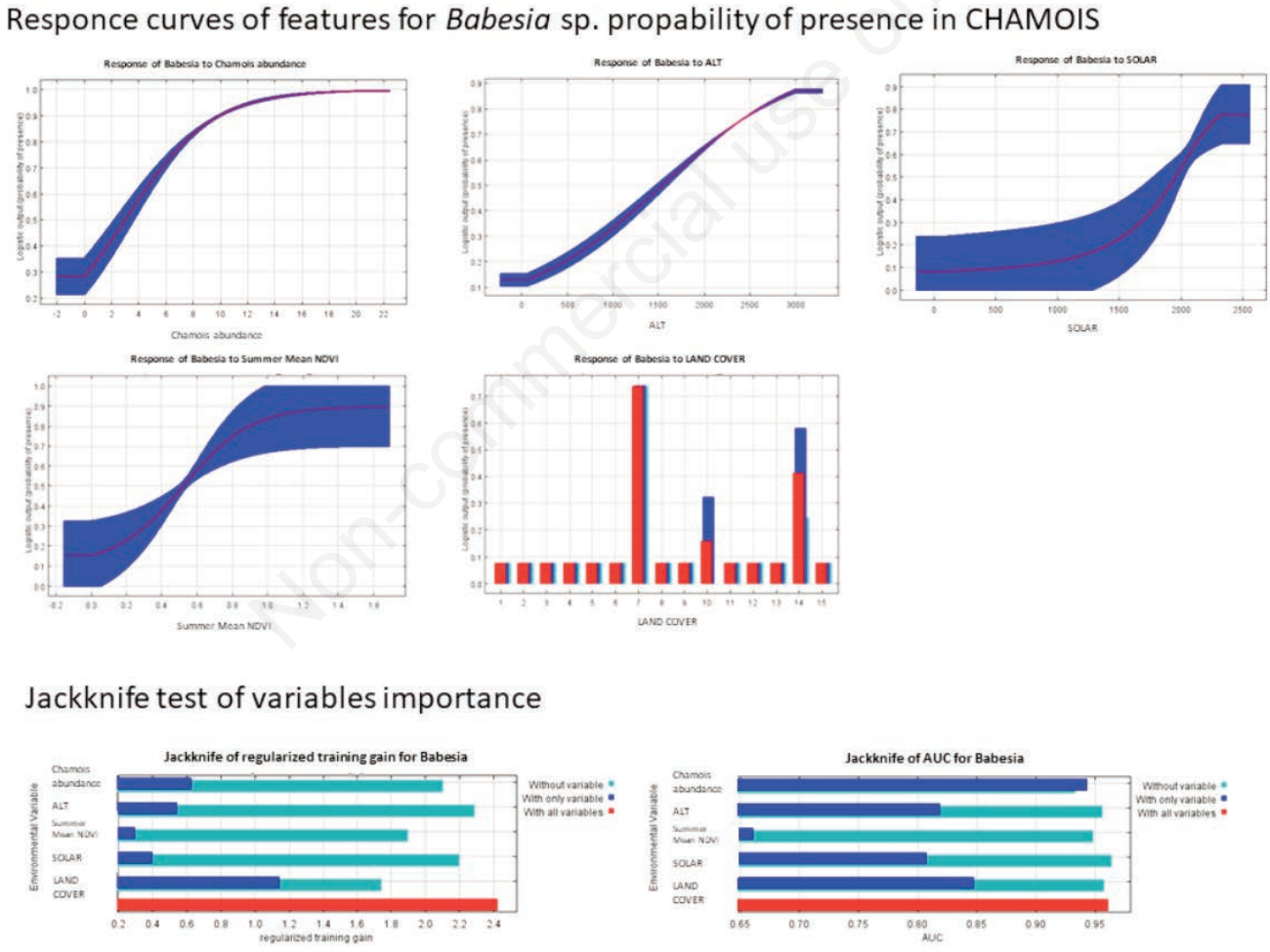

Figure 3. Covariate response curves and Jackknife test of variable importance – Model 2 chamois. Response curves of the selected covariates for *Babesia* spp. occurrence in chamois. Environmental covariates (mean summer NDVI, solar exposure and elevation) and host abundance (chamois) are directly related to higher *Babesia* spp. suitability. Land Cover classes mostly associated to *Babesia* spp. suitability are pastures and Alpine meadows (cat.7 and 14), and shrubs (cat. 10). Jackknife test of variable importance show the impact of each variable on training gain and AUC. Values shown are averages over replicate runs. The environmental variable with highest gain when used in isolation is Land Cover, which therefore appears to have the most useful information by itself, and it is the environmental variable that decreases the gain the most when omitted. Therefore it has most information that is not present in the other variables.

in humans (Ryan *et al.*, 2020). *Babesia* infections are still very much unknown, especially with respect to epidemiology in wildlife hosts and wildlife-related ticks and environments (Yabsley and Shock, 2013). The model covers a variety of different environments: from low-altitude broadleaved forest and agricultural areas, to high-altitude Alpine areas. The resolution of the model was high (1000 m) in order to correctly describe and predict suitable areas for highly selective tick vectors which can persist only in particular areas where a set of environmental characteristics has to occur together with the presence of suitable animal hosts. Biotic and abiotic priors were selected and modelled with regard to the requirements of *I. ricinus*, which is associated with rural environments and wild ungulates (Rizzoli *et al.*, 2014). *I. ricinus* was also the species that was most often recovered (P=99.87%, 95% CI 99.53 – 99.96) from the study region.

All three models fitted the variance of our data well (AUC values ≥0.91). All the covariates included in the models are biologically sound and highly responsive in depicting suitable presence areas. NDVI (with the mean value computed during summer months), slope, solar exposure, altitude and land cover were the most informative environmental features. Each of the three models was best described by different priors, although the response of all variables in each model indicates how areas more exposed to sun light, with a rich vegetation coverage (shrub and broad-leaf forest areas), are the most suitable areas for *Babesia* infections to occur. Pastures and alpine meadows were also found to be suitable for *Babesia* spp., but this is due to the presence in the model of alpine chamois, whose typical home range includes high-altitude open areas. The suitable area for *Babesia* to occur is overall extensive and corresponds to 15% of the territory considered in model development. Human infection occurs mostly in rural or natural environments due to the changes in social and economic behaviours (increase of outdoor activities and re-naturalization of rural areas and territorial expansion of wildlife) (Randolph, 2010; Semenza *et*

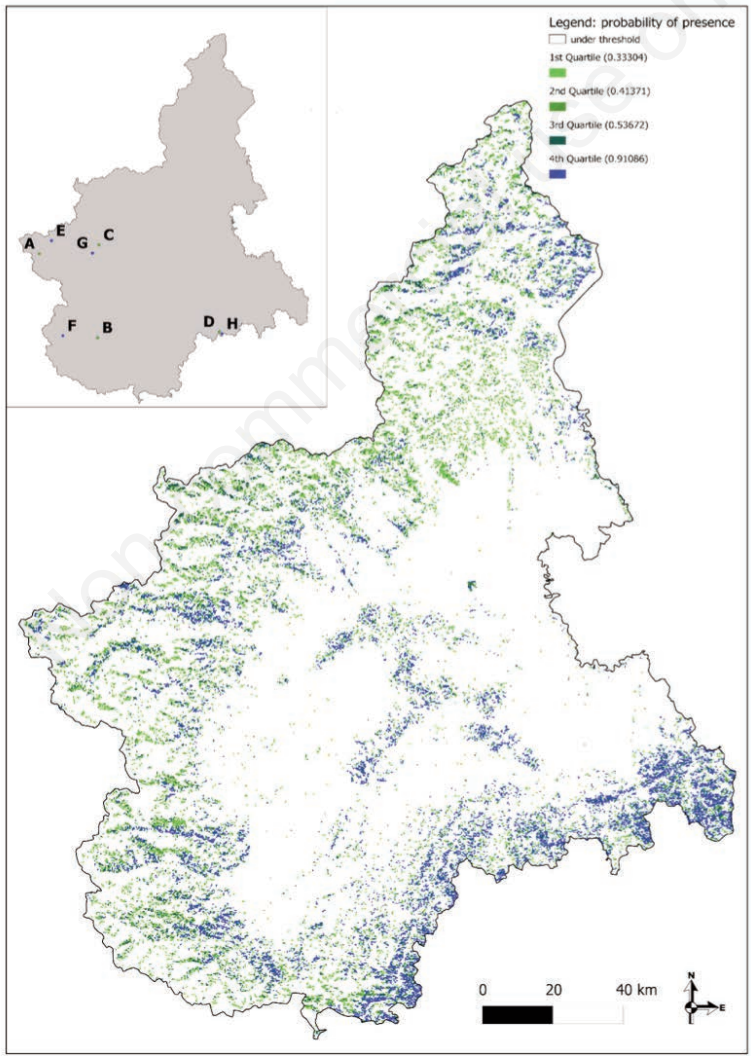

Figure 4. *Babesia* spp. Habitat Suitability Model and empiric validation sampling points. Predicted geographical distribution of the suitable areas for *Babesia* spp. in the three ungulate species. The unsuitable area (below the threshold value of minimum probability of presence) in white, while the suitable area (above the threshold) is divided into quartiles of growing suitability from 1st Q- minimum (light green) to 4th Q- maximum suitability (blue). Tick sampling areas shown in insert (Q2 - from A to D and Q4 - from E to F).

Table 5. Ticks and *Babesia* spp. infection of field model validation.

Site	Gepgraphic identification (coordinates)	Number of larvae [no. of pools]	MIR Babesia (95% CI) [no. of positive pools]	Number of nymphs [no. of positive pools]	MIR Babesia (95% CI) [no. of positive pools]	Number of adults [no. of positive pools]	MIR Babesia (95% CI) [no. of positive pools]	Total MIR (95% CI) [total no. of positive pools]
Q2		224 [38]	26.32% (14.97-42.01) [10]	8 [37]	27.03% (15.4-42.98) [10]	1 [1]	100%(20.65-100) [1]	27.63% (18.84-38.58) [21/76]
A	45°01'03.9"N 6°48'44.8"E	0 [0]	-	2 [2]	0.00% (0.00-65.76) [0]	0 [0]	-	
B	44°28'50.0"N 7°20'47.5"E	22 [8]	0.00% (0.00-32.44) [0]	27 [11]	18.18% (0.51-47.7) [2]	0 [0]	-	
C	45°04'56.2"N 7°20'35.0"E	34 [9]	44.44% (18.87-73.33) [4]	43 [13]	46.15% (23.21-70.86) [6]	1 [1]	100% (20.65-100) [1]	
D	44°31'45.0"N 8°25'26.7"E	168 [21]	28.57% (13.81-49.96) [6]	15 [11]	18.18% (5.14-47.7) [2]	0 [0]	-	
Q4		1,078 [46]	45.65% (32.15-59.82) [21]	163 [37]	40.54% (26.35-56.51) 15]	2 [2]	100% (34.24-100) [2]	44.71% (34.59-55.28) [38/85]
E	45°06'13.3"N 6°55'04.3"E	284 [19]	31.58% (15.36-53.99) [6]	54 [13]	15.38% (4.33-42.23) [2]	1 [1]	100% (20.65-100) [1]	
F	44°29'17.8"N 7°02'22.4"E	0 [0]	-	0 [0]	-	0 [0]	-	
G	45°01'46.7"N 7°17'12.7"E	238 [15]	73.33% (40.05-89.10) [11]	70 [16]	68.75% (44.4-85.84) [11]	1 [1]	100% (20.65-100) [1]	
H	44°31'02.4"N 8°26'53.3"E	556 [12]	33.33% (13.81-60.94) [4]	39 [8]	25% (7.15-59.07) [2]	0 [0]	-	
Total		1,302 [84]	36.90% (27.37-47.58) [31]	250 [74]	33.78% (24.05-45.12) [25]	3 [3]	100% (43.85-100) [3]	36.65% (29.6-44.32) [59/161]

For each geographically marked location (A to H) identified by Model 1 as moderately suitable (Q2) or highly suitable (Q4), the number of larvae, nymphs and adults of Ixodidae ticks collected and the number of homogeneous pools are reported. MIR = minimum infection rate (%); CI = confidence intervals.

Table 6. *Babesia* species identification.

Species	Total no. of positive pools (MIR) [95% CI]	Number of positive pools at Q2 (MIR) [95% CI]	Number of positive pools at Q4 (MIR) [95% CI]	Query coverage	Percent identity	GenBank accession number
Babesia venatorum	38 (23.60%) [17.71-30.73]	14 (18.42%) [11.30-28.58]	24 (28.24%) [19.77-38.58]	100%	99.5 %	KX857480
Babesia microti	4 (2.48%) [0.97-6.21]	1 (1.32%) [0.23-7.08]	3 (3.53%) [1.21-9.87]	100%	100%	FJ608739
Babesia vulpes	4 (2.48%) [0.97-6.21]	1 (1.32%) [0.23-7.08]	3 (3.53%) [1.21-9.87]	100%	100%	KI175166
Babesia capreoli	5 (3.11%) [1.33-7.06]	2 (2.63%) [0.72-9.10]	3 (3.53%) [1.21-9.87]	100%	100%	KU145465
Theileria spp.	8 (4.97%) [2.54-9.50]	3 (3.95%) [1.35-10.97]	5 (5.88%) [2.54-13.04]	100%	100%	MH327771, AJ616717, KX965721

Number of homogeneous tick pools positive for each species. MIR = minimum infection rate (%); CI = confidence intervals.

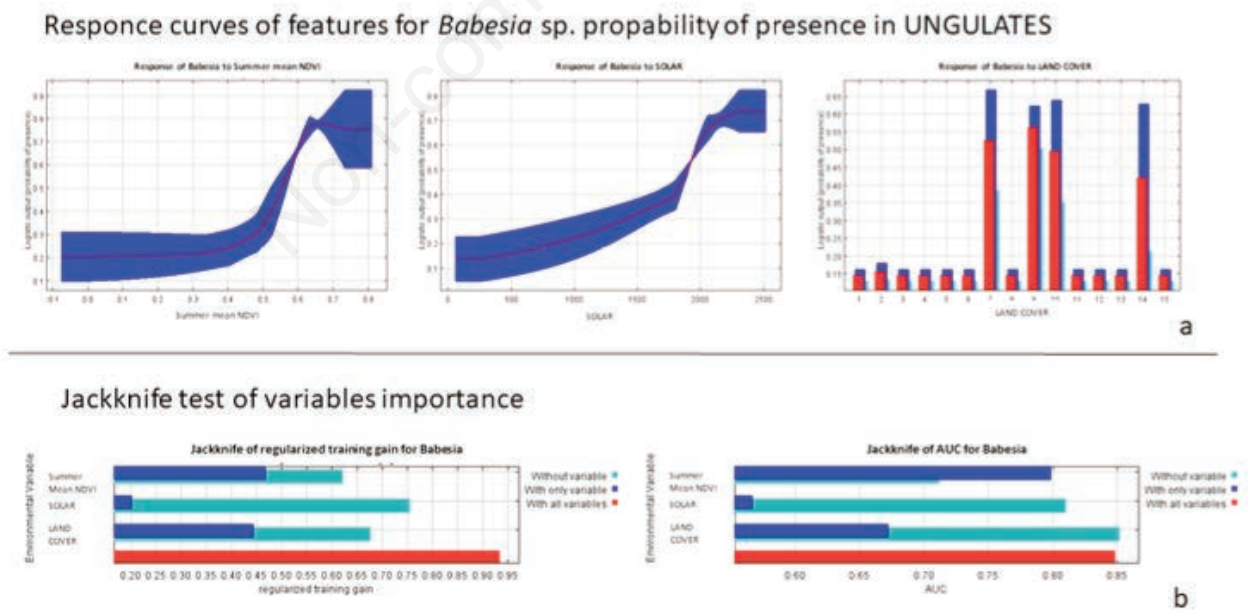

Figure 5. Covariate response curves and Jackknife test of variable importance – Model 3 ungulates. Response curves of the selected covariates for overall *Babesia* spp. occurrence in the three studies species of ungulates. Environmental covariates (mean summer NDVI and solar exposure) are directly related to increased Babesia spp. suitability, while Land Cover classes mostly associated with *Babesia* spp. suitability are pasture (cat.7), broad-leaved forest (cat.9), shrubs (cat. 10), and alpine meadows and pastures (cat.14). Jackknife test of variable importance show the impact of each variable on training gain and AUC. Values shown are averages over replicate runs.

al., 2016). In the last decades, the population of wild ungulates, especially that of roe deer in Italy, has been growing at increasing rates (26% between the years 2000 and 2005; Carnevali *et al.*, 2009). Together with ungulates, the number of ticks has also increased and their geographical range has expanded to include areas at higher latitude and/or altitudes, possibly due to the influence of climate changes on tick habitats (Tälleklint and Jaenson, 1998; Lindgren *et al.*, 2000; Daniel *et al.*, 2003; Estrada-Peña and Venzal, 2006; Materna *et al.*, 2008; Jore *et al.*, 2014). A study by Stainforth *et al.* (2013) documents a decrease between 5% and 10% of the number of winter nights below zero in the study area. Higher winter temperatures favour overwintering and extend the activity season of the vector ticks (Materna *et al.*, 2008) thus increasing the risk of tick-borne diseases.

Field sampling of Ixodidae ticks in the study area was used to validate the *Babesia* spp. occurrence model with empirical data. Results highlighted a significant positive association ($p<0.05$) between *Babesia* spp. MIR in collected ticks and highly suitable areas for parasite occurrence (Q4 areas), thus confirming the ability of the model to correctly predict habitat-suitability for *Babesia* spp. The higher prevalence of infection in the Q4 area than the Q2 areas was confirmed for each of the piroplasmid species identified by sequencing. Most of the species of *Babesia* identified in sampled ticks had been previously reported for the study area. The roe deer used as PP, were infected with *B. venatorum, B. capreoli* and *B. bigemina* (Zanet *et al.*, 2014). Roe deer is the main reservoir host for both *B. venatorum* and *B. capreoli* (Malandrin *et al.*, 2010; Michel *et al.*, 2014), while *B. bigemina,* together with *B. divergens,* is mainly associated with bovine babesiosis (Zintl *et al.*, 2003; Hilpertshauser *et al.*, 2007). *B. capreoli* was the most prevalent species found in red deer used as PP followed by *Theileria* spp., as has been shown previously (Zanet *et al.*, 2014); this species was also detected in the ticks used for empirical validation. All chamois used as PP were infected with *B. capreoli,* as has also been shown previously (Zanet *et al.*, 2014). *B. vulpes* has been reported to infect Red foxes and horses in the same study area (Zanet *et al.*, 2014, 2017). This species is also recognized as a pathogenic in dogs resulting in symptoms of clinical infection ranging from pale mucous membranes, anorexia, apathy to fever with severe macrocytic/hypochromic regenerative anaemia and thrombocytopenia (Guitián *et al.*, 2003; Baneth *et al.*, 2019). Two zoonotic piroplasm species were detected in ticks, namely *B. venatorum and B. microti*. *I. ricinus* is the main vector for both these species (Gray *et al.*, 2002; Bonnet *et al.*, 2007). *B. venatorum* was detected in 6 out of the 8 sampling locations, both in Q2 and Q4 areas. It was not detected only at sites B (Q2) and F (Q4) where *Babesia* spp. were generally detected with lower MIR values or no ticks collected at all. *B. microti* was instead reported only at locations C (Q2) and G (Q4) where *Babesia* spp. with the highest MIR in larvae and nymphs pools were also found.

Conclusions

The use of ecological niche modelling for infectious diseases is a useful tool that allows identifying and prioritizing interventions for disease surveillance, prevention and control. This kind of modelling is highly recommendable for decision makers, managers and politician as it contributes to better use of limited resources.

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