Update on the presence of *Ixodes ricinus* at the western limit of its range and the prevalence of *Borrelia burgdorferi* sensu lato

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Abstract

It is often suggested that due to climate and environmental policy changes, the risk from tick-borne disease is increasing, particularly at the geographical limits of the vector distribution. Our project aimed to determine whether this was true for the risk of Lyme borreliosis in Ireland which is the western-most limit of *Ixodes ricinus*, the European vector of *Borrelia burgdorferi* sensu lato. The availability of a historical data set of tick infection rates compiled in the 1990s represented a unique opportunity as it provided a baseline against which current data could be compared.

Following construction of a spatial predictive model for the presence and absence of *I. ricinus* based on data from 491 GPS locations visited between 2016 and 2019, 1,404 questing nymphs from 27 sites were screened for the presence of *Borrelia* spp. using a TaqMan PCR aimed at the 23S rRNA gene sequence. All positive ticks were further analysed by nested PCR amplification and sequence analysis of the 5S-23S intergenic spacer.

The model indicated that areas with the highest probability of tick presence were mostly located along the western seaboard and the Shannon and Erne river catchments, coinciding with historical high incidence areas of bovine babesiosis, while the infection rate of questing nymphs with *B. burgdorferi* s.l. and the prevalence of the various genospecies have remained surprisingly stable over the last 3 decades. Clear communication of the potential disease risk arising from a tick bite is essential in order to allay undue concerns over tick-borne diseases among the general public.

Keywords: *Borrelia burgdorferi*; *Ixodes ricinus*; spatial predictive model; tick-borne disease; Lyme borreliosis; Ireland; British Isles

Highlights

- The distribution of ticks in Ireland chiefly coincides with historical high incidence areas of bovine babesiosis
- The infection rate of questing nymphs with *B. burgdorferi* s.l. has remained stable over the last 3 decades
- The prevalence of the various genospecies has remained essentially unchanged since the 1990's
- The risk of contracting LB from a tick bite remains low even in woodlands where tick abundance is comparatively high

Introduction

Lyme borreliosis (LB) is a multisystem inflammatory disease caused by spirochaetes in the *Borrelia burgdorferi* sensu lato (s.l.) species complex. Early disease symptoms may include non-specific signs such as fever, lymphadenopathy, myalgia, fatigue and headache. The only pathognomonic symptom is erythema migrans (EM), a spreading skin rash, often with central clearing resembling a bull's eye-pattern which is present in approximately 60-80% of cases (Rizzoli et al., 2011; Stanek et al., 2010). Depending on the *Borrelia* spp. involved, the cardiovascular and nervous systems and joints may also be affected. Patients treated early with appropriate antibiotics usually make a full recovery (Stanek et al., 2010).

B. burgdorferi s.l. is transmitted by 3-host tick species of the genus *Ixodes*, namely *Ixodes ricinus* in Europe, *Ixodes persulcatus* in Eurasia and temperate Asia, and *Ixodes scapularis* and *Ixodes pacificus* in North America.

In the popular media is often claimed that the incidence of LB is increasing throughout its geographical range. However, there is little hard evidence to support this view. The reason for this is that there are no EU-wide surveillance data for LB before January 2019, when Lyme neuroborreliosis was adopted as a notifiable entity at European level (Health Protection Surveillance Centre, 2019). Spatial and historical comparisons prior to this date are challenging because until then the disease was not mandatorily notifiable in many countries. Moreover, there has been a lack of uniformity in the choice of disease symptoms for reporting purposes with some countries describing the incidence of EM, others only including laboratory-confirmed cases, while many studies do not specify their inclusion criteria (Vandekerckhove et al., 2019). Based on these data, estimated annual incidences of LB in Europe range from less than 1 per 100,000 in some countries to over 350 per 100,000 of population in others (Rizzoli et al., 2011). It is generally understood that LB incidence is

highest in the endemic areas of central Europe, decreasing to the southern and northern limits, a geographical distribution that is mirrored by *Borrelia* infection rates in local tick populations (Rizzoli et al., 2011). However, a recent systematic review reported highest infection rates in the north of Europe (Sweden, Norway) followed by countries in central Europe (Switzerland, the Netherlands and Austria), while Italy, Spain and Portugal, continued to have the lowest LB incidences (Vandekerckhove et al., 2019). Moreover, Italy and Luxemburg reported substantial decreases in LB incidence since the early 2000's (Vandekerckhove et al., 2019). Tick infection rates also decrease from eastern to western Europe (Gray et al., 1998; Strnad et al., 2017). All of the high incidence countries reported a continuously increasing trend in LB cases over the last 3 decades (Vandekerckhove et al., 2019) although there was no significant increase in tick infection rates between 1986 to 1993 versus 1994 to 2002 (Rauter and Hartung, 2005) or 2010 to 2016 (Strnad et al., 2017). There is also evidence that, as a result of climate change, the range of LB is expanding into higher latitudes and altitudes. At the same time other, previously suitable areas, may become too warm and/or dry to support *I. ricinus* populations (Lindgren et al., 2000; Gray et al., 2009).

It is important to stress that most of the reports quoted above point out that, depending on the region, LB may be subject to significant under- and overreporting and that in many cases enhanced detection may be due to increased awareness and testing rather than true emergence of the pathogen. Furthermore, the lack of consensus over which diagnostic methods are most reliable and the use of a variety of serological LB tests make it difficult to accurately determine the incidence of disease or to compare the prevalence between decades or regions. Surveillance of LB in Europe is likely to improve with the recent inclusion of Lyme neuroborreliosis on the list of diseases under EU epidemiological surveillance. The argument for using Lyme neuroborreliosis as a key indicator is that it is one of the most frequent early manifestations of disseminated LB in Europe (affecting up to 12% of those

infected) and that, due to the severity of clinical symptoms, it is unlikely to go unreported (Health Protection Surveillance Centre, 2019).

Currently the western range of *I. ricinus* extends as far as Ireland in the north and Portugal in the south. Although nymphs and adults have been reported from Iceland, there is no evidence so far that the tick has established there (Alfredsson et al., 2017). The tick-borne pathogen that has traditionally attracted the most attention in Ireland has not been *B. burgdorferi,* but *Babesia divergens*, a parasitic protozoan that causes bovine babesiosis or redwater fever in cattle. Until the early 1990s *B. divergens* was considered a major economic burden to the Irish livestock industry (Gray and Harte, 1985). However, since then the incidence of redwater fever appears to have drastically declined (Zintl et al., 2014). While there are still foci of infection, particularly in former high incidence areas such as the west and northwest and the Shannon river system, these appear to have contracted in size and number. Anecdotal evidence from farmers indicates that this decline in redwater is associated with a significant reduction in tick numbers (pers. communication). Nevertheless, as has been the case throughout Europe and North America, concern over LB in humans is growing among the Irish public. Since 2014, when Lyme neuroborreliosis first became notifiable in Ireland, between 8 and 21 (on average about 14 cases) cases are reported each year. With regard to the incidence of LB no hard data are available, however it is estimated that there are probably between 10 and 200 cases per annum (0.2 to 4 per 100,000 population) (McKeown and Jackson, 2018). While increased public awareness of the disease was reflected in a sharp rise of Lyme serology requests between 2011 and 2014, the numbers testing positive have remained relatively constant (Health Protection Surveillance Centre, 2019).

One way to determine whether the risk of LB is changing in Ireland is to investigate whether there has been a change in the abundance and/or distribution of ticks and the rate at which they are infected with *Borrelia* spp. Unfortunately, there are no historical tick distribution maps for Ireland. However, there is a substantial body of published data on *Borrelia* infection rates in Irish ticks from the 1990s and early 2000s (Gray et al., 1992, 1995, 1996, 1999, 2000; Kirstein et al., 1997a, 1997b; Pichon et al., 2005). This study sets out to provide the first predictive *I. ricinus* distribution map for Ireland based on field data collected between 2016 and 2019. The map will not only be useful to assess the current risk arising from tick-borne diseases but will also serve as a baseline for future tick distribution studies. In addition, nymphal ticks from 27 sites were screened for the presence of *B. burgdorferi* s.l. spp in order to determine whether there are geographical differences in infection rates and whether they have changed since the earlier surveys took place.

Materials and methods

Field surveys to determine the presence of ticks

Between 2016 and 2019, a total of 154 sites in 25 counties (including 26 in 2016, 58 in 2017, 16 in 2018 and 54 in 2019) were flagged once for the presence of questing ticks using 1x1 m^2 white blanket (Figure 1). In environmentally heterogenous sites several GPS locations (mean distance between locations: 164 meters, Arcmap v10.7, ESRI, USA) were sampled in order to capture different habitats and environmental conditions. Sampling took place between April and August of each year. Field sites included a range of woodland types (deciduous, mixed and coniferous) in national parks, nature reserves and forestry sites, bog habitat, coastal dunes, limestone and acidic grassland, as well livestock farms with rough and improved pasture and hedgerows. At each location, at least 35 5 m^2 drags were performed. All nymphal and adult ticks were identified using taxonomical keys (Estrada-Peña et al., 2017; Hillyard,

1996). Measurements of scutum, setae or spurs to distinguish *I. ricinus* from *Ixodes inopinatus* (Estrada-Peña et al., 2014) were not carried out, however, species identity was confirmed using TaqMan PCR in over a third of collected ticks (37%).

GIS modelling

A spatial predictive model was constructed based on tick presence and absence data collected between 2016 and 2019. For sites where multiple GPS locations were sampled, data from all locations were included in the modelling process increasing the number of data points to 491. Given the binary nature of the response variable (presence or absence), the training dataset was balanced to obtain an equal number of presence and absence points thereby avoiding a biased prediction. The explanatory variables used to build the model are shown in Table 1. All raster files were projected to World Geodetic System (WGS) 1984 and adapted to cover the same geographic extent and to display the same number of pixels in rows and columns.

Two different classification models were explored using VECMAP version 1.5.16209.2382 (Avia-GIS) to determine the best performing algorithm: Boosted Regression Trees (BRT) and Random Forests (RF). After optimisation by manually adjusting the default parameters of the models, both methodologies resulted in similar outputs with regard to validation accuracy metrics (area under the curve (AUC) > 0.9) and variable importance. Spatial prediction was also very similar between both models, but RF resulted in a more gradual distribution of probabilities, which is probably more reflective of what occurs in the field. The RF algorithm was therefore employed to develop the final model. In a variable reduction model (used to identify and discard 50% of the less important variables) 100 points were sampled for each tree, 500 trees were grown and 10 variables at each node were sampled. Then, a prediction forest of 150 points sampled for each tree, 800 trees to grow and 20 variables at each node was created. Variable importance was assessed by the Gini index, which employs an impurity criterion to measure how each variable contributes to the

homogeneity of nodes and leaves in the forest (Breiman et al., 1984). The importance of a variable is based on the Gini index reduction for that variable summed over all nodes.

Selection and preparation of nymphs and DNA extraction

Overall 1,404 nymphs were screened individually for the presence of *Borrelia burgdorferi* s.l. comprising 100 nymphs collected from scrub on limestone pavements (Burren), 49 from a blanket bog (Donegal), 605 from various forests and woodlands and 650 from livestock farms (Supplementary Table 1). With regard to woodland and forests, only sites with at least 40 ticks were included in the PCR screen for statistical reasons. In contrast, all nymphs collected in livestock farms where ticks were generally much less abundant, were included. This was done in order to facilitate a comparison between agricultural and uncultivated land. Ticks collected in 2016 had previously been analysed using a high-throughput PCR-based method (Zintl et al 2017) and were not included in this study.

Prior to DNA extraction, nymphs were immersed twice in 70% ethanol and once in distilled water and blotted dry on tissue paper. They were then transferred individually into homogenisation tubes containing six 2.8 mm stainless Precellys® steel beads (Montigny-Le-Bretonneux, France) and 200 µl of Dulbecco's modified Eagle's medium solution supplemented with 10% foetal bovine serum (Sigma-Aldrich®, USA). Homogenisation was carried out on a Roche MangNA Lyser (Rotkreuz, Switzerland) using 4 rounds at 5000 rpm, for 30 seconds, with 1-minute breaks in between. Subsequently 200 µl lysis buffer and 20 µl proteinase K (both reagents provided in the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) were added and the tubes incubated overnight at 56 ºC with shaking (100 rpm). The following day, DNA was extracted using the QIAamp DNA Mini Kit following the manufacturers' recommendations.

TaqMan PCR assays for the detection of B. burgdorferi *s.l. DNA and confirmation of tick species identity*

DNA extracts were screened for the presence of *B. burgdorferi* s.l. DNA using a TaqMan PCR protocol adapted from Michelet et al. (2014). The primers and probe targeted a 73bp region in the 23S ribosomal RNA gene (Table 2). The reaction mix contained 1x TaqMan Environmental Master Mix 2.0 (Applied Biosystems TM, USA), 300 nM forward primer, 100 nM reverse primer, 200 nM probe and 5 µl DNA template in an overall reaction volume of 20 µl. Each sample was analysed in duplicate. Only samples that gave rise to a double amplification were considered positive.

In addition to the PCR screen for the presence of *Borrelia* spp., a second TaqMan PCR targeted at the internal spacer region 2 (ITS2) of *I. ricinus* was performed (Table 2). This was done in order to confirm the tick species identity and as a control for successful DNA extraction. For this PCR assay the reaction mix contained 1x FastStart Universal Probe Master (ROX) (Roche Diagnostic GmbH, Germany), 300 nM of each primer, 100 nM of the probe and 5 µl DNA template in an overall reaction volume of 20 µl. No duplicates were included in this assay. For both reactions the amplification protocol consisted of 10 minutes at 95 ºC followed by 40 cycles at 95 ºC (10 seconds) and 60 ºC (1 minute). Positive controls consisting of a 410bp synthetic DNA construct with binding sites for all primers and probes (Eurofins) and negative controls containing no template DNA were included in all PCR assays.

Nested conventional PCR for the identification of B. burgdorferi *s.l. species in TaqMan-positive samples*

All samples that were positive by TaqMan PCR were further analysed by nested PCR using a protocol that amplified a 225 bp fragment of the spacer region between the 23S and 5S

ribosomal RNA genes (Table 2) (Rijpkema et al., 1995). Both PCR assays were performed in a total volume of 50 µl containing 5 µl DNA template (1st PCR) or 5 µl primary PCR product (in the nested PCR), 1x PCR Master Mix (Thermo Fisher Scientific Inc., USA), 0.2 mM of each deoxynucleoside triphosphate, 0.24 µM of the forward and reverse primers, and 1.25 U DreamTaq DNA Polymerase. Negative controls (master mix without a DNA template) were included in each assay. To assess the sensitivity of the TaqMan assay, 70 samples that were negative by TaqMan PCR were also tested using the nested PCR. None of these samples yielded PCR amplicons.

All PCR products were purified using the High Pure PCR Clean-up Micro Kit (Roche Diagnostics GmbH, Germany) and sequenced from both directions using the nested PCR primers (GATC Biotech, Eurofins Genomics, Germany). Following alignment of the forward and reverse sequences and deletion of the primer sequences, genospecies were identified by comparing them against published *B. burgdorferi* s.l. sequences on GenBank using Clustal Omega.

Statistical analysis

Model performance was assessed by Cohen's kappa, AUC, sensitivity and specificity. These accuracy metrics were obtained by comparing observed and predicted results using the Out-of-the-bag (OOB) data, which are the data not used to build the tree in each model. The kappa statistic is an observer agreement measure calculated by comparing the 'observed accuracy' against the 'expected accuracy' (Landis and Koch, 1977). It can be particularly useful to evaluate models built with unbalanced training datasets or multi-class problems. According to Landis and Koch (1977) kappa is considered almost perfect for K= 0.81 to 1, substantial= 0.61 to 0.8, moderate = 0.41 to 0.6, fair = 0.21 to 04, slight = 0 to 0.2, or poor < 0. AUC is obtained by generating the receiver operating characteristic (ROC) curve, which is

a plot of sensitivity against 1-specificity (Altman and Bland, 1994). A model is considered excellent for AUC = 0.9 to 1, good for 0.8 to 0.9, fair for 0.7 to 0.8, poor for 0.6 to 0.7 and unacceptable for 0.5 to 0.6.

The proportion of infected nymphs collected from woodland and farms in 2017 to 2019 was compared to the proportion of infected nymphs collected in the 1990s based on 95% confidence intervals for proportions and the z statistic (Moore and McCabe, 1999).

Results

Species identity and distribution of ticks in Ireland

Ticks were found in 61 (40%) of the 154 sites that were visited between 2016 and 2019. Overall 3,818 ticks were collected including 3,486 nymphs, 183 adult male and 149 adult female ticks. The presence of larvae was only recorded if no other tick life cycle stage was collected. Based on morphology all ticks were identified as *I. ricinus*.

The predictive map for the distribution of *I. ricinus* (Figure 2) indicates that the most suitable areas are located along the western seaboard of the island, including counties Donegal and Sligo in the North West, Mayo and Galway in the west and parts of Kerry and West Cork in the South West. In addition, the Lough Erne and parts of the river Shannon catchments were highlighted as potentially suitable for ticks. In the rest of the country there are a number of isolated foci of predicted tick presence. Model performance was as follows: Cohen's Kappa: 0.75, AUC: 0.93, sensitivity: 0.80 and specificity: 0.90. The most important variables according to the Mean Decrease of Gini Index were: Normalized Difference Vegetation Index (NDVI) during May, NDVI during April, minimum temperature during May, NDVI during November, average rainfall during November, NDVI during December, NDVI during March, minimum temperature during summer season, NDVI during January,

average temperature during the summer season, habitat, minimum temperature during December, NDVI during February, elevation and annual total number of rain days.

Prevalence of B. burgdorferi *s.l. and identity of the genospecies present*

Of the total number of nymphs screened for the presence of *B. burgdorferi* s.l. (n=1,404), 129 (9.2%) were positive by TaqMan PCR (95% confidence interval (CI): 7.7 to 10.7%). Ticks collected from woodland (n=650) had infection rates of 14.2% (CI: 11.4 to 17.0%) which was significantly higher than infection rates in nymphs collected on livestock farms (n=650, prevalence: 5.4%; CI: 3.8 to 7.3%) ($z = 5.26$, $p<0.0001$). However, the higher average tick infection rate in woodlands was chiefly due to comparatively high prevalences detected in Muckross Demesne , Killarney National Park (n=272, prevalence: 24.6%, CI: 19.5 to 29.7%). Nymphs collected from limestone pavements (n=100, prevalence: 4.0%, CI: 0.2 to 7.8 %) and bogland (n=49, prevalence: 6.1%, CI: 0-12.8%) had similar infection rates to those from farms but were not included in this analysis because the numbers of ticks analysed from these sites were comparatively low.

In 4 sites where very high tick densities allowed for the separate analysis of tick infection rates from different locations within the site (Killarney National Park, Newcastle wood, and 2 farms in counties Kerry and Leitrim), there were no significant differences between the locations and the 95% confidence intervals overlapped. A full breakdown of screening results for the various field collection sites is provided in Supplementary table 1.

Thirty-nine of the tick samples (30.2% of the total) that were positive for *B. burgdorferi* s.l according to TaqMan PCR were negative by nested PCR, and a further 3.9% (n=5) yielded amplicons but poor sequencing data. Of the remaining isolates, 35 (41.2%) were identified as *B. valaisiana*, 30 (35.3%) as *B. burgdorferi* s.s., 18 (21.2%) as *B. garinii* and 2 (2.4%) as *B.*

afzelii. *Borrelia valaisiana* was the most common genospecies identified in both woodland and farmland (Table 3), followed by *B. burgdorferi* sensu stricto (s.s.), in woodland and *B. garinii* on farms. Only *B. valaisiana* and *B. burgdorferi* s.s. were identified in ticks from limestone pavement and bogland, however the overall number of infected ticks from both of these sites was very low.

All but three isolates matched previously published sequences on GenBank (Table 4). The remaining three, which were identified as *B. burgdorferi* s.s., were identical to each other but differed from the most similar sequence in the database (Z77166) by a single base pair. This sequence has been logged on GenBank under accession number LC524008.

Discussion

It is often claimed that due to changes in climate and environmental policy and their effects on habitat suitability and host availability, the risk from tick-borne disease is increasing particularly at the geographical limits of the vector distribution. This study aimed to investigate changes in the range of *I. ricinus* and its infection rates with *B. burgdorferi* s.l. at the western-most limit of the tick species. During the course of the study almost 4,000 questing nymphs and adult ticks were collected all of which were identified as *I. ricinus*, indicating that this remains the only tick species present in Ireland that can be collected by blanket dragging/flagging. *Dermacentor reticulatus* and *Haemaphysalis punctata* which have been described from several locations in the UK (Estrada-Peña et al., 2017; Medlock et al., 2017, 2018), and are widespread throughout Europe and *I. inopinatus*, which is sympatric with *I. ricinus* in parts of southern and central Europe (Hauck et al., 2019), appear to remain absent from Ireland. It is important to remember, however, that *D. reticulatus* can peak

during winter and early spring. As a result populations, if they do occur in Ireland, may have been overlooked.

According to the predicted distribution map the probability for *I. ricinus* to be present is high all along the western seaboard and the Shannon and Erne river catchments in the midlands. While there are no historical tick distribution maps for comparison, many of the predicted hotspots coincide with former high incidence areas of bovine babesiosis (Zintl et al., 2014), indicating that the decline in bovine babesiosis that has been recorded over the last four decades is chiefly due to improved on-farm control measures rather than a change in environmental suitability.

As a three-host tick, *I. ricinus* spends more than 98% of its life off the host in the environment which explains why environmental parameters, namely vegetation, temperature and rainfall were identified by the model as the most important predictor variables of tick presence. The strict requirement of all life cycle stages for a high relative humidity (>80%) is well documented. Even in the mild, humid climate characteristic for Ireland these requirements are mostly met along the west coast where precipitation is highest and in habitats with dense vegetation and permanently moist leaf litter. Interestingly land cover (as specified by the CORINE dataset) only ranked 24th. The reason for this may be that due to the data resolution of 25 hectares used by the CORINE maps land-cover areas below 25 hectares are not included. As has been suggested by various environmental experts this may not be sufficient to correctly identify typical Irish habitats as the Irish landscape is highly fragmented (Thompson, 2019). It is important to stress, of course, that where no tick hosts are available, ticks will be absent even if habitat and climate are favourable.

The *B. burgdorferi* s.l. infection rate in nymphs collected from woodland ranged between 2.4 (0-7% C.I.) and 24.6% (19.5-29.7% C.I.). By comparison, Kirstein et al. (1997a) who analysed 686 nymphs collected in 6 woodland sites throughout Ireland in 1995 reported infection rates ranging between 4 (0.2-7.8% C.I.) and 17.9% (12-23.8% C.I.). Other surveys carried out in some of the same and one additional woodland site during the 1990s and early 2000's also recorded remarkably similar nymph infection rates to the ones reported in the present study (ranging between 6.1%, CI: 4-8.2% and 18.5%, CI: 14.7-22.2%) (Gray et al., 1992, 1996, 1999, 2000; Kirstein et al., 1997b; Pichon et al., 2005). Figure 3 shows past and present *B. burgdorferi* s.l. infection rates in ticks by county indicating that areas of high infection pressure and the risk of being bitten by an infected tick have not materially changed in the last 30 years. Similar results have been reported in the rest of Europe with two meta-analyses concluding that the prevalence of *B. burgdorferi* s.l. in questing ticks has remained reasonably constant over the last 3 decades even though the screening methods that are being used have become increasingly more sensitive (Rauter and Hartung, 2005; Strnad et al., 2017). The TaqMan qPCR employed in the present survey would also have been more sensitive than the methods used in the previous Irish studies (indirect fluorescence assay and single conventional PCR). In this context it is important to stress that just over 30% of samples that were positive by TaqMan PCR were negative by nested PCR. It is unclear whether this was because the TaqMan assay, which is targeted at a gene locus that is duplicated in each genome (the 23S rRNA gene), was more sensitive than the nested PCR which amplifies a single copy locus (the variable spacer region between 2 repeated copies of the 23S and 5S ribosomal genes) or whether the TaqMan PCR detected *Borrelia* spp. outside the *B. burgdorferi* s.l. group which would not have been picked up by the nested PCR because the tandem duplication of the 5S and 23S rRNA genes and the spacer region between the copies is unique to the *B. burgdorferi* s.l. group (Rijpkema et al., 1995; Schwarz et al

1992). However, even if some non-*B. burgdorferi* s.l. species were present it is unlikely they would have accounted for such a large proportion of TaqMan-positive ticks. Previous studies that used primers that were able to detect *B. miyamotoi* reported infection rates ranging between 0.25 and 1.01% in Irish ticks (Lambert et al., 2019; Pichon et al., 2015). High-throughput microfluidic system analysis of 197 *I. ricinus* collected in 2016 from 26 sites throughout Ireland failed to detect any *Borrelia* spp. in the relapsing fever group (Zintl et al., 2017).

As reported in previous studies (Gray et al., 1995) infection rates in nymphs from agricultural habitats were considerably lower than in nymphs collected in woodland. The same was true for ticks collected from bogland and limestone pavement indicating that larvae in these sites chiefly feed on hosts that are not competent reservoirs for *B. burgdorferi* s.l. such as deer, sheep or cattle, while larvae in woodland may have access to a larger range of host species including potential *B. burgdorferi* s.l. reservoirs such as wood mice and passerine birds. A limited variety of *B. burgdorferi* s.l. reservoir hosts in bogland and on limestone pavement was also indicated by the reduced diversity of *Borrelia* genospecies. However, the overall number of positive ticks identified in these sites was also low. In both woodland and farms *B. valaisiana* was the predominant species. The same was true for nymphs investigated in the 1990s surveys (Kirstein et al., 1997a, 1997b) except on two occasions in Killarney National Park and Connemara National Park (Gray et al., 2000) where *B. garinii* was somewhat more frequently detected. The clinical significance of *B. valaisiana* is still unclear as it has only been identified in a less than a handful of clinical cases (Stanek and Reiter, 2011). Interestingly this species, which seems to be chiefly carried by birds, is most commonly detected in ticks from western Europe with infection rates decreasing towards central and eastern Europe. In contrast *B. afzelii*, a rodent-associated genospecies that causes chronic skin conditions, is generally more common in ticks collected in eastern Europe (Strnad et al.,

2017) although high prevalences have also been reported from parts of Scandinavia and Scotland (Hvidsten et al., 2015; James et al., 2014). *Borrelia afzelii* was only rarely detected in this study and in the previous ones (Gray et al., 2000; Kirstein et al., 1997a, 1997b; Pichon et al., 2005). *Borrelia garinii* which causes neuroborreliosis and is mostly associated with birds (although some serotypes infect rodents) and *B. burgdorferi* s.s., which is also associated with neuroborreliosis as well as arthritis and carried by rodents, are important pathogens both in Ireland and in continental Europe (Rauter and Hartung, 2005). *Borrelia garinii* was detected in almost 35% of nymphs from farms and *B. burgdorferi* s.s. in almost 38% of nymphs from woodland. Similar infection rates of the two genospecies in nymphs were reported in the previous studies with *B. garinii* ranging from approximately 20 to almost 45% in Killarney, and up to 47% in Connemara and *B. burgdorferi* s.s. detected in between 20 (Killarney) and 31% (Portumna) (Kirstein et al., 1997a, 1997b), although the 2 more recent studies had reported lower infection rates with the latter genospecies (none in Connemara and 12% in Killarney; Gray et al., 2000; Pichon et al., 2005). The high combined prevalence of bird-associated genospecies provides further evidence that birds are important hosts for *I. ricinus* larvae in Ireland as has been suggested previously (Gray, 1998). No additional genospecies such as *B. lusitaniae*, *B. bavariensis*, *B. spielmanii*, *B. finlandensis* or *B. bissettiae* were identified in the present study. However, no attempts were made to amplify additional gene loci.

Conclusions

While evidence from the veterinary and farming communities suggests that the population of *I. ricinus* and the most important veterinary tick-borne pathogen in Ireland, *B. divergens*, has been declining for several decades, public concern about LB and potential co-infections with other zoonotic tick-borne pathogens has been on the increase. This study indicates that the

distribution of *I. ricinus* in Ireland, the infection rate of questing nymphs with *B. burgdorferi* s.l. and the prevalence of the various genospecies have remained essentially unchanged since the 1990s. Interestingly, similarly stable *B. burgdorferi* s.l. infection rates in *I. ricinus* ticks have also been described for many other countries in Europe (Strnad et al., 2017, Rauter and Hartung, 2005) which is surprising given that policy-driven changes in land use together with climate change are likely to have pronounced direct and indirect effects on host availability and diversity. It may be the case that the host/vector/*Borrelia* dynamics are in fact more robust than anticipated and that environmental changes will have to become more dramatic before they manifest in radically changed tick distributions and infection rates.

The highest *B. burgdorferi* s.l. prevalences of around 25% were recorded in a couple of locations in Killarney National Park, which is probably a reflection of the comparatively high diversity of fauna in the area (see Kirstein 1997). Infection rates in most other sites were substantially lower. Moreover at least one third of infected nymphs carried *B. valaisiana* which is not a primary human pathogen. Consequently the risk of contracting LB from a tick bite remains low even in woodlands where tick abundance is comparatively high. Nevertheless there are small foci of LB-infected ticks in sites that are used for leisure activity and on farmland, close to human habitations. Therefore prompt removal of ticks that have attached is always recommended (Health Protection Surveillance Centre, 2019). It is important that accurate information on the potential risk arising from tick-borne diseases is communicated clearly to the public in order to alleviate unwarranted concerns as to the risks associated with outdoor activities.

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References

Alfredsson, M., Olafsson, E., Eydal, M., Unnsteinsdottir, E.R., Hansford, K., Wint, W., Alexander, N., Medlock, J.M., 2017. Surveillance of *Ixodes ricinus* ticks (Acari: Ixodidae) in Iceland. Parasit Vectors. 10, 466-477. DOI: 10.1186/s13071-017-2375-2.

Altman, D.G., Bland, J.M., 1994. Diagnostic tests 3: receiver operating characteristic plots. BMJ. 309, 188. DOI: 10.1136/bmj.309.6948.188

Breiman, L., Friedman, J.H., Olshen, R.A., Stone, C.J., 1984. Classification and Regression Trees. Wadsworth International Group, Belmont.

Danielova, V., Rudenko, N., Daniel, M., Holubova, J., Materna, J., Golovchenko, M., Schwarzova, L., 2006. Extension of *Ixodes ricinus* ticks and agents of tick- borne diseases to mountain areas in the Czech Republic. Int. J. Med. Microbiol. 296 (Suppl 40), 48-53. DOI: 10.1016/j.ijmm.2006.02.007

Estrada- Peña, A., Mihalca, A.D., Petney, T.N., 2017. Ticks of Europe and North Africa, a guide to species identification. Springer International Publishing, Cham, Switzerland

Estrada-Peña, A., Nava, S., Petney, T., 2014. Description of all the stages of *Ixodes inopinatus* n. sp. (Acari: Ixodidae). Ticks Tick Borne Dis. 5, 734-43. DOI: 10.1016/j.ttbdis.2014.05.003.

Gray, J.S., 1998. The ecology of ticks transmitting Lyme borreliosis. Exp Appl Acarol. 22, 249–58.

Gray, J.S., Harte, L.N., 1985. An estimation of the prevalence and economic importance of clinical bovine babesiosis in the Republic of Ireland. Ir Vet J, 39, 75-78.

Gray, J.S., Kahl, O., Janetzki, C., Stein, J., 1992. Studies on the ecology of lyme disease in a deer forest in County Galway, Ireland. J Med Entomol. 29, 915–20. DOI: 10.1093/jmedent/29.6.915

Gray, J.S., Kahl, O., Janetzki, C., Stein, J., Guy, E., 1995. The spatial distribution of *Borrelia burgdorferi*-infected *Ixodes ricinus* in the Connemara region of County Galway, Ireland. Exp Appl Acarol. 19, 163-72. DOI: 10.1007/bf00046288

Gray, J.S., Schönberg, A., Postic, D., Belfaiza, J., Saint-Girons, I., 1996. First isolation and characterisation of *Borrelia gari*nii, agent of Lyme borreliosis from Irish ticks. Ir J Med Sci. 165, 24-6. DOI: 10.1007/bf02942796

Gray, J.S., Kahl, O., Robertson, J.N., Daniel, M., Estrada-Peña, A., Gettinby, G., Jaenson, T.G., Jensen, P., Jongejan, F., Korenberg, E., Kurtenbach, K., Zeman, P., 1998. Lyme

borreliosis habitat assessment. Zentralbl Bakteriol. 287, 211-28. DOI: 10.1016/s0934-8840(98)80123-0

Gray, J., Kirstein, F., Robertson, J.N., Stein, J., Kahl, O., 1999. *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks and rodents in a recreational park in south-western Ireland. Exp Appl Acarol. 23, 717–29. DOI: 10.1023/a:1006233700194

Gray, J.S., Robertson, J.N., Key, S., 2000. Limited role of rodents as reservoirs of *Borrelia burgdorferi* sensu lato in Ireland. Eur J Epidemiol. 16, 101–3. DOI: 10.1023/a:1007642908546

Gray, J.S., Dautel, H., Estrada-Peña, A., Kahl, O., Lindgren, E., 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip Perspect Infect Dis. 2009:593232. DOI: 10.1155/2009/593232.

Hauck, D., Springer, A., Pachnicke, S., Schunack, B., Fingerle, V., Strube, C., 2019. *Ixodes inopinatus* in northern Germany: occurrence and potential vector role for *Borrelia* spp., Rickettsia spp., and *Anaplasma phagocytophilum* in comparison with *Ixodes ricinus*. Parasitol Res. 118:3205-3216. DOI: 10.1007/s00436-019-06506-4.

Health Protection Surveillance Centre, 2019. Primary prevention and surveillance of Lyme Borreliosis in Ireland. Report of the Lyme Borreliosis Subcommittee of the Scientific Advisory Committee of the Health Protection Surveillance Centre. https://www.hpsc.ie (accessed 18 March 2020)

Hillyard, P.D., 1996. Ticks of North West Europe. In: Barnes RSK, Crothers JH, editors. Synopses of the British fauna. No 52. Linnean Society of London and The Estuarine and Coastal Sciences Association. Montford Bridge: Field Studies Council Publications.

Hvidsten, D., Stordal, F., Lager, M., Rognerud, B., Kristiansen, B.E., Matussek, A., Gray, J., Stuen, S., 2015. *Borrelia burgdorferi* sensu lato-infected *Ixodes ricinus* collected from vegetation near the Arctic Circle. Ticks Tick Borne Dis. 6, 768-73. DOI: 10.1016/j.ttbdis.2015.07.002.

James, M.C., Gilbert, L., Bowman, A.S., Forbes, K.J., 2014. The heterogeneity, distribution, and environmental associations of *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. Front Public Health. 2, 129-139. DOI: 10.3389/fpubh.2014.00129.

Kirstein, F., Rijpkema, S., Molkenboer, M., Gray, J.S., 1997a. The distribution and prevalence of *B. burgdorferi* genomospecies in *Ixodes ricinus* ticks in Ireland. Eur J Epidemiol. 13, 67–72. DOI: 10.1023/a:1007360422975

Kirstein, F., Rijpkema, S., Molkenboer, M., Gray, J., 1997b. Local variations in the distribution and prevalence of *Borrelia burgdorferi* sensu lato genomospecies in *Ixodes ricinus* ticks. Appl Environ Microbiol. 63, 1102–6.

Lambert, J.S., Cook, M.J., Healy, J.E., Murtagh, R., Avramovic, G., Lee, S.H., 2019. Metagenomic 16S rRNA gene sequencing survey of *Borrelia* species in Irish samples of *Ixodes ricinus* ticks. PLoS One. 14, e0209881. DOI: 10.1371/journal.pone.0209881

Landis, J.R., Koch, G.G., 1977. The Measurement of Observer Agreement for Categorical Data. Biometrics 33, 159-174.

Lindgren, E., Tälleklint, L., Polfeldt, T., 2000. Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick *Ixodes ricinus*. Environ Health Perspect. 108, 119-23. DOI: 10.1289/ehp.00108119

McKeown, P., Jackson, S., 2018. Lyme disease awareness day. Disease surveillance report of HPSC, Ireland. Epi-Insight 19, 1-5

Medlock, J.M., Hansford, K.M., Vaux, A.G.C., Cull, B., Abdullah, S., Pietzsch, M.E., Wall, R., Johnson, N., Phipps, L.P., 2017. Distribution of the tick *Dermacentor reticulatus* in the United Kingdom. Med Vet Entomol. 31, 281-288. DOI: 10.1111/mve.12235

Medlock, J.M., Hansford, K.M., Vaux, A.G.C., Cull, B., Pietzsch, M.E., Gillingham, E.L., Johnson, N., Phipps, L.P., 2018. Has the red sheep tick, *Haemaphysalis punctata*, recently expanded its range in England? Med Vet Entomol., 32(4):473-480. DOI: 10.1111/mve.12335

Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., 2014. High- throughput screening of tick-borne pathogens in Europe. Front Cell Infect Microbiol. 24, 1–13. DOI: 10.3389/fcimb.2014.00103

Moore, D.S., McCabe, G.P., 1999. Introduction to the practice of statistics. 3rd edition. WH Freeman & Company, New York

Morán Cadenas, F., Rais, O., Jouda, F., Douet, V., Humair, P.F., Moret, J., Gern, L., 2007. Phenology of *Ixodes ricinus* and infection with *Borrelia burgdorferi* sensu lato along a north- and south-facing altitudinal gradient on Chaumont Mountain, Switzerland. J Med Entomol. 44, 683–693. DOI: 10.1603/0022-2585(2007)44[683:poirai]2.0.co;2

Pichon, B., Rogers, M., Egan, D., Gray, J., 2005. Blood-meal analysis for the identification of reservoir hosts of tick-borne pathogens in Ireland. Vector Borne Zoonotic Dis. 5, 172-80. DOI: 10.1089/vbz.2005.5.172

Rauter, C., Hartung, T., 2005. Prevalence of *Borrelia burgdorferi* sensu lato genospecies in *Ixodes ricinus* ticks in Europe: a metaanalysis. Appl Environ Microbiol. 71, 7203-16. DOI: 10.1128/AEM.71.11.7203-7216.2005

Rijpkema, S.G., Molkenboer, M.J., Schouls, L.M., Jongejan, F., Schellekens, J.F., 1995. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. J Clin Microbiol. 33, 3091-5.

Rizzoli, A., Hauffe, H., Carpi, G., Vourc, H.G., Neteler, M., Rosa, R., 2011. Lyme borreliosis in Europe. Euro Surveill. 16, pii: 19906.

Schwartz, J.J., Gazumyan, A., Schwartz, I., 1992. rRNA gene organization in the Lyme disease spirochete, *Borrelia burgdorferi*. J Bacteriol. 174, 3757-65. DOI: 10.1128/jb.174.11.3757-3765.1992

Stanek, G., Fingerle, V., Hunfeld, K.P., Jaulhac, B., Kaiser, R., Krause, A., Kristoferitsch, W., O'Connell, S., Ornstein, K., Strle, F. and Gray, J., 2010. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect. 17, 69-79. DOI: 10.1111/j.1469-0691.2010.03175.x

Stanek, G., Reiter, M., 2011. The expanding Lyme Borrelia complex—clinical significance of genomic species? Clin Microbiol Infect. 17, 487-493. DOI: 10.1111/j.1469-0691.2011.03492.x

Strnad, M., Hönig, V., Růžek, D., Grubhoffer, L., Rego, R.O.M., 2017. Europe-wide meta-analysis of *Borrelia burgdorferi* sensu lato prevalence in questing *Ixodes ricinus* ticks. Appl Environ Microbiol. 83, pii: e00609-17. DOI: 10.1128/AEM.00609-17

Thompson, S., 2019. Ireland needs more detailed land-use maps. The Irish Times https://www.irishtimes.com/news/science/ireland-needs-needs-more-detailed-land-use-maps-1.4010070 (accessed 18 March 2020)

Vandekerckhove, O., De Buck, E., Van Wijngaerden, E., 2019. Lyme disease in Western Europe: an emerging problem? A systematic review. Acta Clin Belg. 18, 1-9. DOI: 10.1080/17843286.2019.1694293

Zintl, A., McGrath, G., O'Grady, L., Fanning, J., Downing, K., Roche, D., Casey, M., Gray, J.S., 2014. Changing incidence of bovine babesiosis in Ireland. Ir Vet J. 67, 19-26. DOI: 10.1186/2046-0481-67-19

Zintl, A., Moutailler, S., Stuart, P., Paredis, L., Dutraive, J., Gonzalez, E., O'Connor, J., Devillers, E., Good, B., OMuireagain, C., De Waal, T., Morris, F., Gray, J., 2017. Ticks and Tick-borne diseases in Ireland. Ir Vet J., 70, 4-15. DOI: 10.1186/s13620-017-0084-y

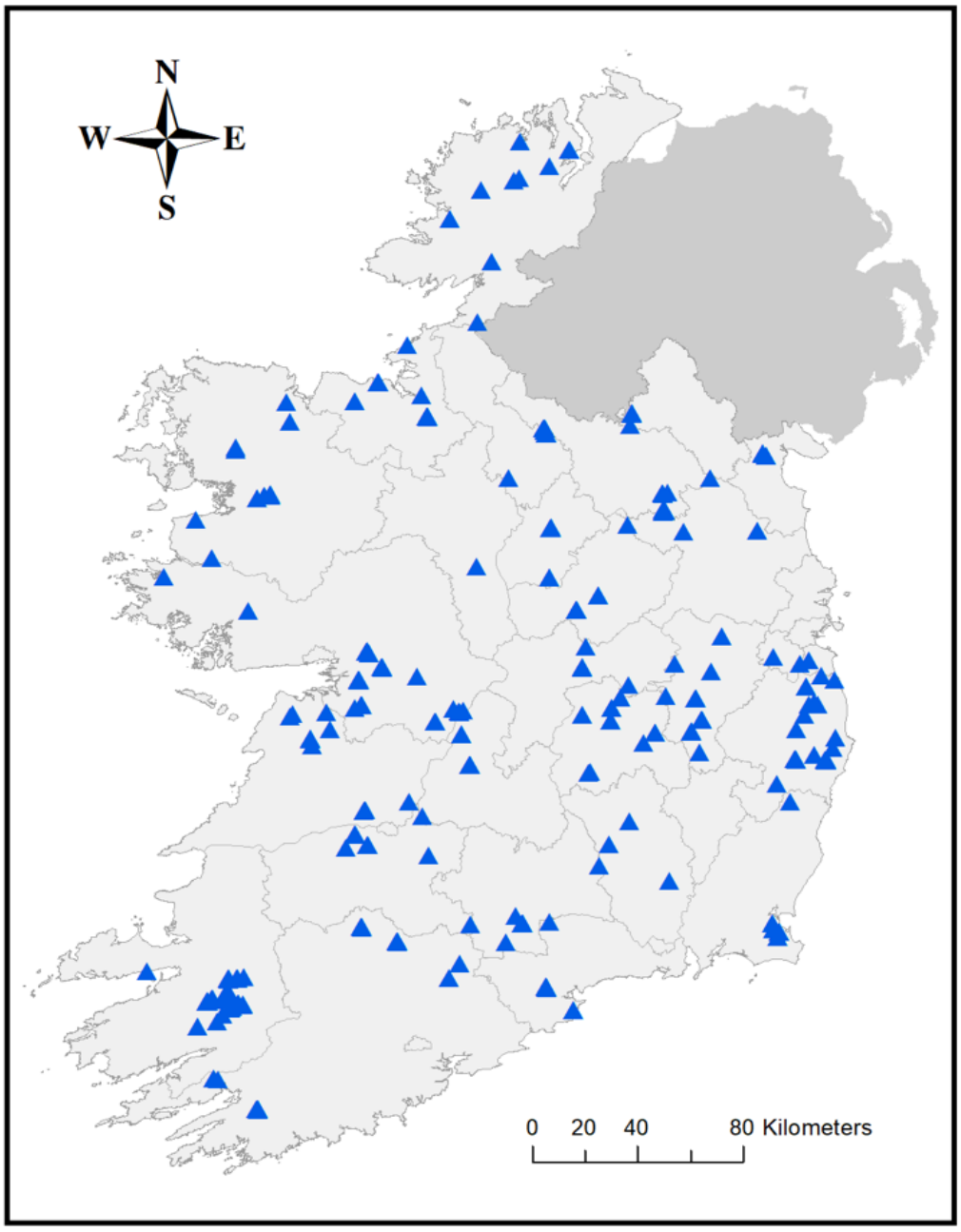

Figure 1: Location of sampling sites visited between 2016 and 2019

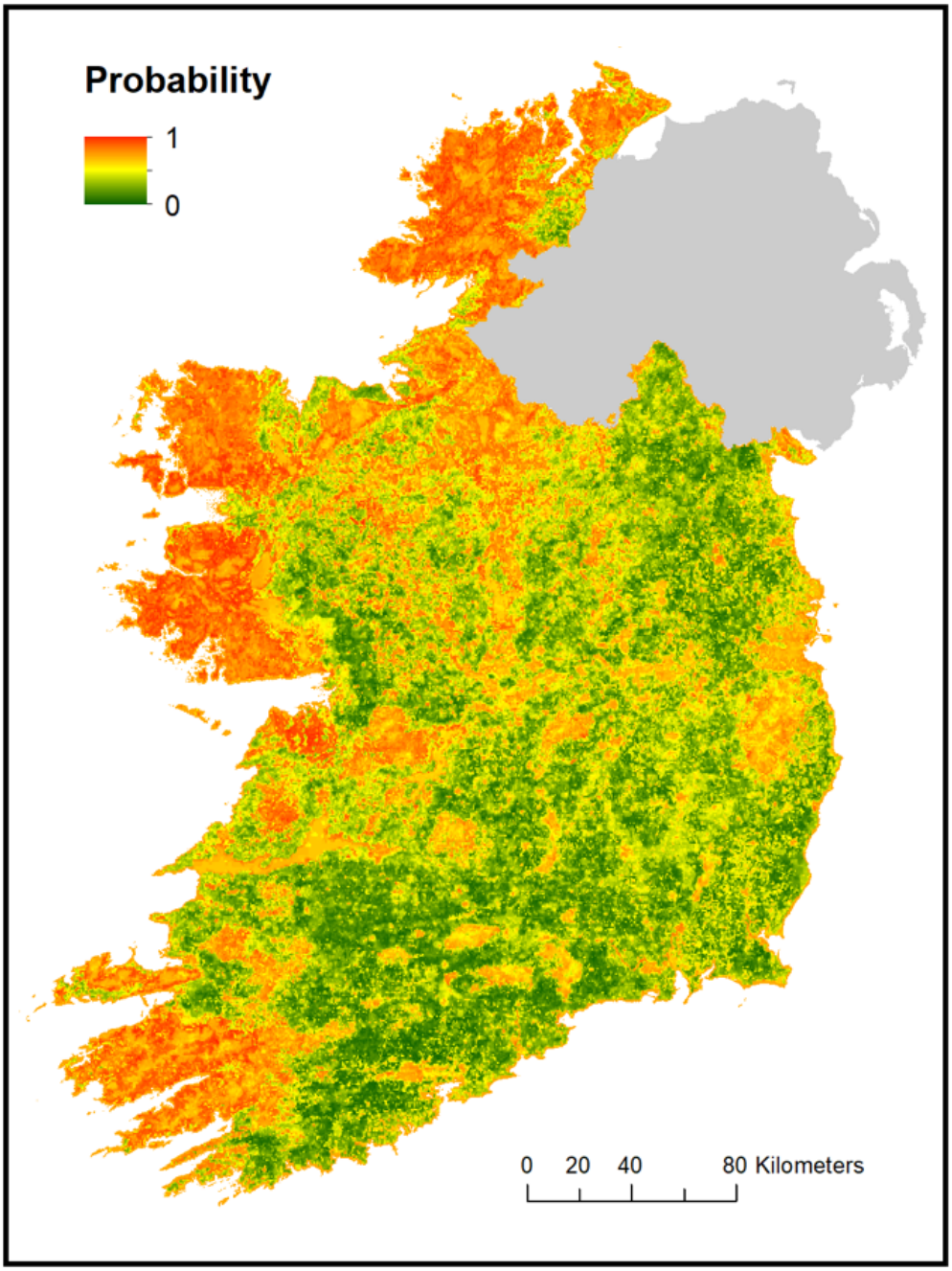

Figure 2: Spatial distribution of predicted probabilities for the presence of *I. ricinus* in Ireland

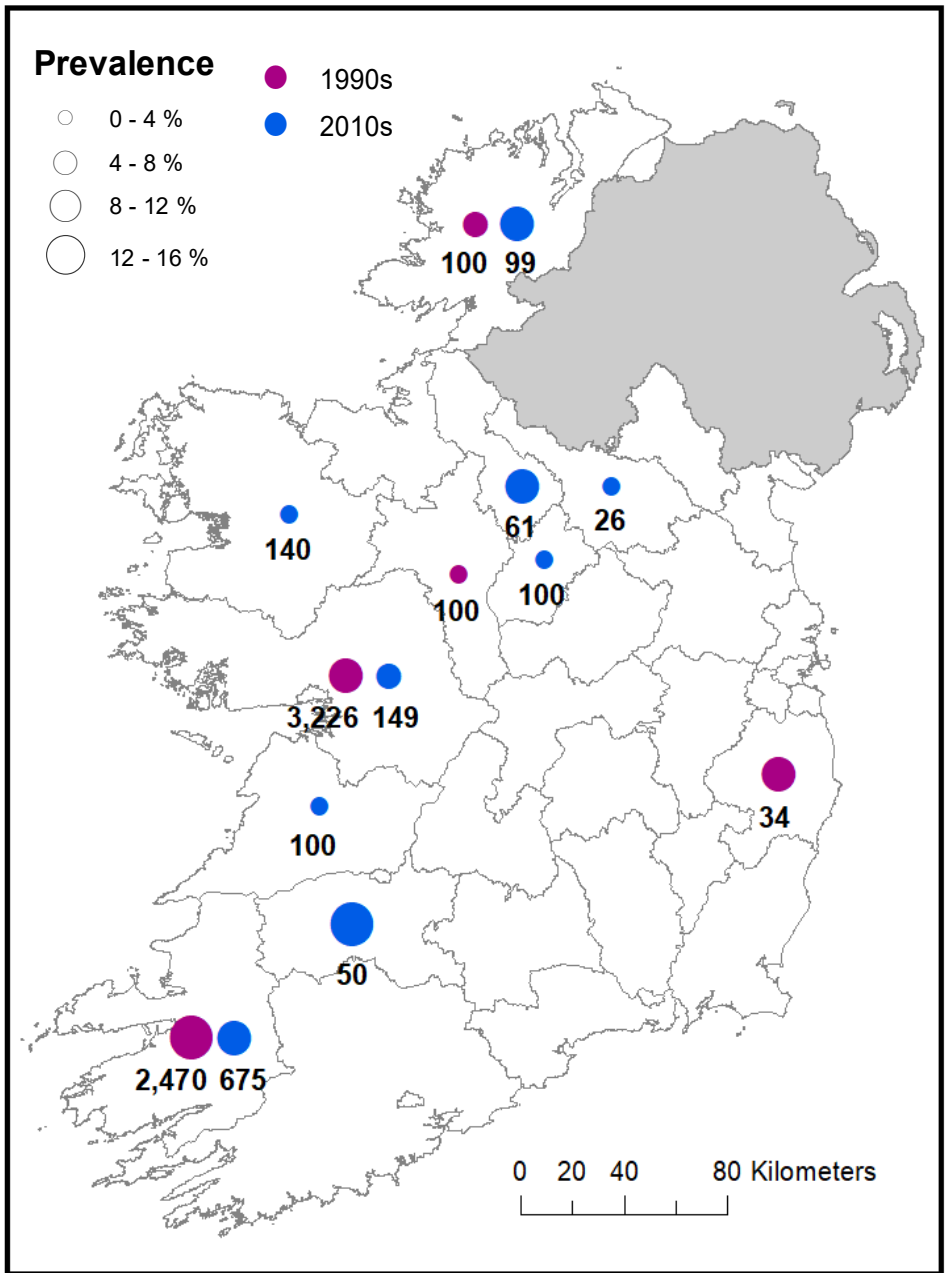

Figure 3: *Borrelia burgdorferi* s.l. infection rates (by county) in *I. ricinus* nymphs analysed in the 1990s and in 2016-2019 (figures beside the circles indicate the overall number of nymphs screened)

Table 1: Environmental variables employed for modelling

Variable	Description	Source and resolution
Precipitation period 2010-2015	Averages of annual, seasonal and monthly total rainfall (mm), and annual total number of rain-days (daily rainfall ≥0.2 mm)	Met Éireann (1 x 1 km), interpolated values
Temperature period 2010-2015	Average, maximum and minimum annual, seasonal and monthly temperatures	MODIS/Terra Land Surface Temperature/Emissivity Monthly L3 Global (6 x 6 km)
Soil type and soil drainage	National Soils Database	EPA (scale 1:250,000)
Habitat	National Habitat Indicator Map	Teagasc (25 x 25 m)
Land Cover	2012 CORINE land cover dataset	EPA (25 ha minimum mapping unit)
Elevation	National Elevation Map	UCD Maps and GIS library. Processed by CVERA (25 x 25 m)
Vegetation period 2010-2015	Monthly average of normalized difference vegetation index (NDVI)	MODIS/Terra Vegetation Indices 16-Day L3 Global (250 x 250 m)

EPA: Environmental Protection Agency; UCD: University College Dublin; CVERA: Centre for Veterinary Epidemiology and Risk Analysis; MODIS: Moderate-Resolution Imaging Spectroradiometer; Seasons: winter (January to March inclusive), spring (April to June inclusive), summer (July to September inclusive), autumn (October to December)

Table 2 Primers and probes used in the TaqMan and nested PCR protocols

Target	**Primer sequence**	**Product size (bp)**	**Reference**
23S rRNA of *B. burgdorferi* s.l. (TaqMan PCR)	Fw: 5'GAGTCTTAAAAGGGCGATTTAGT Rev: 5'CTTCAGCCTGGCCATAAATAG Probe: 5'AGATGTGGTAGACCCGAAGCCGAGT	73	Michelet et al., 2014
ITS 2 of *I. ricinus* (TaqMan PCR)	Fw: 5'GAAACTCGATGGAGACCTG Rev: 5'ATCTCCAACGCACCGACGT Probe: 5'TTGTGGAAATCCCGTCGCACGTTGAAC	77	
23-5S spacer region of *B. burgdorferi* s.l. (nested PCR)	1st Fw: ACCATAGACTCTTATTACTTTGAC 1st Rev: TAAGCTGACTAATACTAATTACCC	380	Rijpkema et al., 1995
	2nd Fw: ACCATAGACTCTTATTACTTTGACCA 2nd Rev: GAGAGTAGGTTATTGCCAGGG	225	

Table 3 Combined *B. burgdorferi* s.l. infection rates in different habitats and prevalence (%) of genospecies

	Nymph infection rate (numbers tested)	*B. afzelii*	*B. burgdorferi* s.s.	*B. garinii*	*B. valaisiana*
Limestone pavement	4.0 (100)		66.7		33.3
Bogland	6.0 (49)		66.7		33.3
Woodland	14.2 (605)	1.9	37.7	17.0	43.4
Farm	5.5 (650)	3.9	23.1	34.6	38.5

Table 4 Molecular characterisation of *B. burgdorferi* s.l. isolates

	Accession Nº	**n**	**Reference**
B. afzelii	Z77173	2	Wittenbrink et al, 1996
B. burgdorferi s.s.	Z77166	26	Wittenbrink et al, 1996
	XXX	3	This study
	Z77172	1	Wittenbrink et al, 1996
B. garinii	GQ387031	8	Gern et al 2010
	JX909889	3	Wilhelmsson et al 2013
	GQ387030	3	Gern et al 2010
	GQ387029	2	Gern et al 2010
	GQ387032	2	Gern et al 2010
B. valaisiana	U78150	23	Wang et al 1997
	JX909999	7	Wilhelmsson et al 2013
	U78148	3	Wang et al 1997
	U78147	1	Wang et al 1997
	JX910027	1	Wilhelmsson et al 2013

Supplementary Table 1: Collection sites, numbers of nymphs screened, and prevalence of *B. burgdorferi* s.l. and genospecies

County	Name & short description	Lat	Long	Date	No of ticks analysed	% positive (95% confidence interval)	*B. burgdorferi* genospecies
Clare	Slieve carron (Burren): Limestone pavement with short scrub Nature reserve	53.0763	-8.99871	2017	50	2.0 (0-5.9)	*B. valaisiana* (n=1)
Clare	Lough Bunny: Limestone pavement on the lake shore National Park	53.0177	-8.97661	2017	50	6.0 (0-12.6)	*B. burgdorferi* s.s. (n=2)
Donegal	Barra Bog: Lowland blanket bog with isolated shrubs Nature reserve	54.948	-8.12595	2017	49	6.1 (0-12.8)	*B. burgdorferi* s.s. (n=2) *B. valaisiana* (n=1)
Donegal	Kincrum: Mixed woodland	54.8433	-8.30725	2017	50	10.0 (1.7-18.3)	*B. burgdorferi* s.s. (n=2) *B. valaisiana* (n=1)
Galway	Portumna forest Park: Commercial coniferous woodland with patches of deciduous woodland	53.0831	-8.24303	2017	50	8.0 (0.5-15.5)	*B. afzelii* (n=1) *B. burgdorferi* s.s. (n=1) *B. garinii* (n=1) *B. valaisiana* (n=1)
Galway	Derrycrag wood: Deciduous woodland Nature reserve	53.0471	-8.37964	2017	42	2.4 (0-7.0)	*B. burgdorferi* s.s. (n=1)
Kerry	Small mixed woodland bordering a dairy farm	52.030533	-9.464483	2018	40	5.0 (0-11.8)	*B. valaisiana* (n=2)
Kerry	Killarney National Park: Deciduous woodland				272	24.6 (19.5-29.7)	*B. burgdorferi* s.l. (n=2) *B. burgdorferi* s.s. (n=12) *B. garinii* (n=7) *B. valaisiana* (n=17)
Including:	Dundag	52.0132	-9.50657	2017	54	16.7 (6.8-26.6)	
	Muckross House	52.018283	-9.499950	2017	80	36.3 (25.8-46.8)	
	The Demesne	52.060444	-9.520083	2018	138	21.0 (14.2-27.8)	
Longford	Newcastle wood: Commercial coniferous woodland with patches of deciduous woodland				97	5.2 (0.8-9.6)	*B. burgdorferi* s.s. (n=1) *B. valaisiana* (n=2)
Including:	Edge of the path	53.56266	-7.72583	2019	63	3.2 (0-7.5)	
	Middle of the woodland	53.5633	-7.7255	2019	34	8.8 (0-18.3)	
Mayo	Letterkeen woods: Coniferous woodland	54.0082	-9.54955	2017	29	3.5 (0-10.1)	*B. burgdorferi* s.s. (n=1)
Mayo	Oldhead wood: Deciduous woodland Nature reserve	53.776	-9.778	2017	25	4.0 (0-11.7)	*B. garinii* (n=1)
	Farms						
Cavan	Dairy farm			2019	26	0	

Galway	Dairy farm	2019	57	3.5 (0-8.3)	*B. afzelii* (n=1) *B. garinii* (n=1)
Kerry	Dairy farm	2018	70	5.7 (0.3-11.1)	*B. garinii* (n=2)
Kerry	Dairy farm	2018	120	10 (4.6-15.4)	*B. burgdorferi* s.l. (n=1) *B. burgdorferi* s.s. (n=3) *B. garinii* (n=2) *B. valaisiana* (n=4)
Including:	site 1: stone wall on the perimeter of the property		40	15 (3.9-26.1)	
	site 2: stone wall and ditch separating two fields		40	12.5 (2.3-22.8)	
	site 3: farm track verges		40	2.5 (0-7.3)	
Kerry	Beef farm	2018	125	3.2 (0.1-6.3)	*B. burgdorferi* s.s. (n=2) *B. garinii* (n=1)
Kerry	Mixed beef and sheep farm	2018	48	8.3 (0.5-16.1)	*B. garinii* (n=1)
Leitrim	Beef farm	2019	54	3.7 (0-8.7)	*B. valaisiana* (n=2)
Including:	site 1: middle of a water-logged pasture		18	0	
	site 2: hedge separating two fields		36	5.6 (0-13.1)	
Leitrim	Beef farm	2019	4	25 (0-67.4)	*B. valaisiana* (n=1)
Leitrim	Beef farm	2019	3	0	
Limerick	Dairy farm	2019	50	14 (4.4-23.6)	*B. garinii* (n=2) *B. valaisiana* (n=3)
Longford	Beef farm	2019	3	0	
Mayo	Beef farm	2019	83	0	
Mayo	Beef farm	2019	1	0	
Mayo	Mixed beef and sheep farm	2019	2	0	
Offaly	Mixed beef and sheep farm	2019	3	0	
Tipperary	Dairy farm	2019	1	0	