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Symposium article

Abstract

Spatial heterogeneity in parasite susceptibility and exposure is a common source of confounding variation in disease ecology studies. However, it is not known whether spatial autocorrelation acts on immunity at small scales, within wild animal populations, and whether this predicts spatial patterns in infection. Here we used a well-mixed wild population of individually recognised red deer (*Cervus elaphus*) inhabiting a heterogeneous landscape to investigate fine-scale spatial patterns of immunity and parasitism. We noninvasively collected 842 faecal samples from 141 females with known ranging behaviour over two years. We quantified total and helminth-specific mucosal antibodies and counted propagules of three gastrointestinal helminth taxa. These data were analysed with linear mixed models using the Integrated Nested Laplace Approximation (INLA), using a Stochastic Partial Differentiation Equation approach (SPDE) to control for and quantify spatial autocorrelation. We also investigated whether spatial patterns of immunity and parasitism changed seasonally. We discovered substantial spatial heterogeneity in general and helminth-specific antibody levels and parasitism with two helminth taxa, all of which exhibited contrasting seasonal variation in their spatial patterns. Notably, *Fasciola hepatica* intensity appeared to be strongly influenced by the presence of wet grazing areas, and antibody hotspots did not correlate with distributions of any parasites. Our results suggest spatial heterogeneity may be an important factor affecting immunity and parasitism in a wide range of study systems. We discuss these findings with regards to the design of sampling regimes and public health interventions, and suggest

that disease ecology studies investigate spatial heterogeneity more regularly to enhance their results, even when examining small geographic areas.

Introduction

Parasite infection in the wild is extremely spatially heterogeneous. The scale at which spatial variation acts depends on the host and parasite being studied, and even fine-scale environmental heterogeneity may influence the spatial epidemiology of human diseases (Murdock *et al.* 2017). However, the spatial ecology of disease is most often considered in terms of large-scale patterns (e.g. Murray *et al.*, 2018) and using occurrence or prevalence data, which is less informative than intensity, particularly for macroparasites. In addition, due to practical considerations, many studies investigating spatial variation in wild animals compare several discrete populations rather than sampling across a continuous, mixing population (e.g. Downs *et al.* 2015; Cheynel *et al.* 2017). Alternatively, some studies rely on opportunistic convenience sampling, which can produce an inaccurate representation of disease processes and bias estimates of infection prevalence due to their non-random sampling in space (Nusser *et al.* 2008). As a result, little is known about fine-scale patterns of susceptibility and exposure, and how they influence spatial patterns of infection, in wild animals.

Identifying the relevant spatial scale for disease processes such as susceptibility and exposure is important, as quantifying spatial trends at different scales can introduce uncertainty at best, and can profoundly affect the conclusions drawn at worst (Gilligan *et al.* 2007; Vidal-Martínez *et al.* 2010; Lachish and Murray 2018). For example, Lyme disease risk correlates positively with biodiversity at the within-forest level, but the reverse is true between forests (Wood and Lafferty 2013). An understanding of spatial processes is therefore crucial for designing public health interventions (Caprarelli and Fletcher 2014) and sampling regimes (Nusser *et al.* 2008; Vidal-Martínez *et al.* 2010; Lachish and Murray 2018). A deeper understanding of fine-scale spatial variation in disease processes could also inform patterns seen over wider distances (Murdock *et al.* 2017; Pawley and McArdle 2018). In addition, if immunity and parasitism vary over short distances, infection-oriented studies of wild populations could be affected by greater degrees of spatial dependence than previously considered, which can affect inference. When spatial autocorrelation is not considered, the type I error rate may be inflated due to inflated covariance of explanatory and/or response variables emerging from geographic proximity (Pawley and McArdle 2018).

Spatial variation in immunity can originate from gradients in abiotic conditions such as temperature (Laughton *et al.* 2017) or in biotic factors such as prey availability (Becker *et al.* 2018). Spatial variation in parasitism will arise in part as a result of this immune heterogeneity owing to variation in susceptibility, clearance, and tolerance (Jolles et al. 2015), as well as from abiotic factors affecting parasite transmission (e.g. sunlight; Parsons *et al.* 2015) or from

variation in abundance of secondary hosts or vectors (Sol *et al.* 2011; Olsen *et al.* 2015). In addition, conspecific density can influence resource availability, immune investment, and parasite exposure (Wilson *et al.* 2004; Downs *et al.* 2015; Ezenwa *et al.* 2016). We therefore expect to see considerable spatial variation in both immunity and parasitism in heterogeneous environments (Becker et al. 2019); where gradients are steep and mixing is minimal, this variation should occur over short distances. A recent study in wild mice (*Mus musculus*) demonstrated high between-site immune heterogeneity, but with extensive variation in the degree of within-site differentiation, suggesting short-range spatial dependence (Abolins *et al.* 2018). However, few studies have examined how both immunity and parasitism vary continuously across space within wild animal populations, so it is unclear to what degree spatial variation in parasitism in the wild originates from immune-mediated processes rather than from environmental factors affecting exposure. Finally, spatial patterns are rarely static, and may change over time (Hawkins 2012), yet seasonal or annual changes in these spatial patterns are rarely examined.

The red deer (*Cervus elaphus*) is a large land mammal closely related to the American wapiti (*Cervus canadensis*) whose distribution covers much of Europe. The relationship between red deer disease and their spatial behaviour is important to pathogen spillover, as this species carries a plethora of parasites that can infect humans and livestock (Bohm *et al.* 2007; Brites-Neto *et al.* 2015) and which they can vector between farms and distribute through the landscape (Chintoan-Uta *et al.* 2014; Qviller *et al.* 2016). The wild red deer living in the North Block of the Isle of Rum in Scotland are individually recognised and regularly censused, providing detailed information on each individual's life history and ranging behaviour (Clutton-Brock *et al.* 1982). These censuses have previously been used to uncover important roles of the environment and spatial behaviour in influencing individuals' phenotypes (Stopher *et al.* 2012; Froy *et al.* 2018). Longitudinal noninvasive faecal sampling of the population has revealed a high prevalence of several gastrointestinal helminth parasites including strongyle nematodes, the liver fluke *Fasciola hepatica*, and the tissue nematode *Elaphostrongylus cervi* (Albery, Kenyon, *et al.* 2018). The life cycle of strongyle nematodes is direct, while *F. hepatica* must infect *Galba truncatula* water snails (Taylor *et al.* 2016), and *E. cervi* infects a range of land snails (Mason 1989). Their mucosal antibodies (IgA) have also been quantified by faecal ELISA, offering a measure of immune investment (Albery, Watt, *et al.* 2018). Both helminth intensity and IgA concentrations are affected by deer reproductive investment and fluctuate seasonally (Albery, Watt, *et al.* 2018). However, the spatial distributions of these immune and parasite measures have yet to be investigated.

In this study, we used regular census data and noninvasive faecal samples from the deer population to investigate how individuals' spatial behaviour was associated with immunity and

parasitism at fine spatial scales. We incorporated spatial autocorrelation structures in order to investigate how this affected model fit, to identify hotspots of immunity and infection, and to quantify the spatial scale at which our data were autocorrelated. We also allowed spatial autocorrelation structures to vary seasonally. We expected that accounting for spatial autocorrelation would improve model fit, and that this would be a more effective way of investigating spatial trends than separating the population into discrete arbitrary subpopulations as was previously done to control for spatial variation (Huisman et al. 2016). We also predicted that individuals living in different areas of the study system would exhibit notably different antibody levels and parasite intensities. Finally, we predicted that *F. hepatica* and *E. cervi* intensity would be influenced by the habitats of their secondary hosts – particularly that *F. hepatica* would be more common in wetter areas (Olsen *et al.* 2015).

Methods

Study system and sampling regime

The study population is located in the north block of the Isle of Rum, Scotland (57°N, 6°20′W; Figure 1). The sampling area measures ~4 km north-south and ~3 km west-east (total area ~12.7km^2). The most intensely sampled area consists of a river running from south to north along a valley, flanked by hills on either side, and an extended ranging area around the coast to the east, close to the sea. Peat bogs and *Juncus* marshland comprise much of the southern and central areas of the valley, while the hills are dominated by wet and dry heath and *Molinia* grassland. In the north, moving seaward, the landscape is dominated by *Agrostis* and *Festuca* grassland, followed by sandy dunes and beaches. The study population is wild and unmanaged, and is censused five times a month for eight months of the year (see Clutton-Brock *et al.*, 1982). During censusing, one of two predetermined routes is walked or driven through the study area and individuals' locations (to the nearest 100 metres) are noted. The northern part of the study area hosts the highest population density, with most deer centred around the high-quality grazing near the mouth of the river and the land around the coast to the east (Figure 1). Annual home ranges are highly repeatable from year to year (Stopher *et al.* 2012).

The deer reproductive cycle ("deer year") spans from the start of the calving season, May 1st, until April 30th the following year. Samples were collected as previously described (Albery, Kenyon, *et al.* 2018), on a seasonal basis during 7 two-week trips in August ("summer"), November ("autumn") and April ("spring") between April 2016 and April 2018 inclusive. Note that our dataset included a sampling trip from April 2016, which was part of the deer year beginning in May 2015, with no accompanying summer and autumn trips from this

reproductive cycle. In the study period, 842 faecal samples were collected noninvasively from 141 individually known adult females aged 3 and above. Parasite propagule counts and antibody ELISA quantification were carried out on these samples as previously described (Albery, Kenyon, *et al.* 2018; Albery, Watt, *et al.* 2018). Parasites included strongyle nematodes (order: Strongylida), the common liver fluke *Fasciola hepatica* and the red deer tissue nematode *Elaphostrongylus cervi*. Our two antibody measures were total mucosal IgA levels ("total IgA") and anti-*Teladorsagia circumcincta* L3 larval antigen IgA ("anti-Tc IgA"). The former is taken as an indicator of general investment in mucosal immunity, while the latter gives a measure of specific anti-strongyle IgA response which is thought to be more indicative of protective immunity against strongyles (Watt *et al.* 2016; Albery, Watt, *et al.* 2018). There was not enough faecal matter in all samples to quantify all variables; final sample sizes are displayed in Table 1. Using the census data, each individual's mean easting and northing over the deer year was taken as their average location. This was taken to be a better indication of an individual's spatial behaviour than the location at which the faecal sample itself was collected. We subdivided the study area into six approximate subpopulations based on each individual's average location (Huisman *et al.* 2016). These locations and subpopulations are displayed in Figure 1.

Statistical analysis

Statistical analysis was carried out using the Integrated Nested Laplace Approximation (INLA). INLA is a deterministic Bayesian approach which is increasingly being used for analysis of spatial data (Zuur *et al.* 2017). Models were fitted in R version 3.5 (R Core Team 2018) using the linear modelling package R-INLA (Rue and Martino 2009; Martins *et al.* 2013). We constructed five generalised linear mixed models (GLMMs) for each response variable, each featuring different combinations of fixed and spatial random effects. The distinguishing components of these model sets are outlined below and displayed in Table 1.

Our five response variables included integer counts per gram of three parasite propagules following a negative binomial distribution (strongyles, *F. hepatica* and *E. cervi*) and Gaussian-distributed optical densities of two mucosal antibodies (total IgA and anti-Tc IgA). Antibody levels were corrected for collection effects as previously described, by taking the residuals from a linear model including raw antibody OD as a response variable and including day of collection, time of collection and extraction session as explanatory variables (Albery, Watt, *et al.* 2018). In our main GLMMs, explanatory variables included: Deer year (categorical with three levels: 2015, 2016, and 2017); Season (categorical with three levels: Summer, Autumn, and Spring); Age (continuous, in years); Reproductive status (categorical variable with three levels: No Calf, Calf Died, and Calf Survived; see Albery, Watt, *et al.* (2018) for definitions); Subpopulation (categorical, six levels). All models included individual ID as a random effect.

INLA allows incorporation of a spatially distributed random effect to account for spatial autocorrelation (Lindgren *et al.* 2011). This uses a stochastic partial differentiation equation (SPDE) approach to approximate the continuous random field using a triangulated mesh of connected discrete locations (Lindgren and Rue 2015). The mesh we used for the spatial random effect is displayed in Figure 1. The random effect can be plotted in 2D (giving the "spatial field" of variation) to investigate hot- and coldspots of the response variable, and the kappa/range parameters can be extracted to investigate the distance at which autocorrelation fades in space. It is also possible to allow multiple spatial fields within a single model, by assigning separate fields to different categories or by linking fields with correlation structures to investigate spatiotemporal variation. The underlying mathematics of INLA and associated spatial/spatiotemporal models have been extensively discussed elsewhere, and such models are increasingly being used to examine spatiotemporal trends (e.g. fisheries ecology (Cosandey-Godin *et al.* 2015)); see http://www.r-inla.org for more examples.

We constructed a set of competing models for each response variable. Each model set contained five models, resulting in 25 models total. Our base model set (model set 1) included year, season, age, and reproductive status as fixed effects, similar to models previously used to investigate associations between reproduction, immunity, and parasitism (Albery, Watt, *et al.* 2018). Model set 2 added subpopulation as a fixed effect to investigate whether this explained any variation and to examine the value of analysing continuous populations using discrete subdivisions (Figure 1). Model set 3 added a spatially distributed SPDE random effect, rather than the subpopulation fixed effect, to control for and quantify spatial autocorrelation. In model set 4, this spatial field was allowed to vary between seasons (summer, autumn, and spring), and model set 5 allowed correlation between these seasonal fields. To allow spatial fields to correlate, we used an "exchangeable" model, where all fields in the model were correlated by the same value (ρ) rather than e.g. following an autoregressive process through time. We elected not to fit different spatial fields across years as our number of replicates was small for detecting annual variation. We also had no *a priori* hypotheses concerning spatial differences between years; splitting up the spatial field into individual sampling trips (field:season:year) would cut down the sample size considerably for each field, reducing the likelihood of picking up spatial patterns; and we have only one season (Spring) from the first year of collection, so the years are unlikely to be comparable.

For each response variable, the five fitted models were compared using Deviance Information Criterion (DIC). A change in 2 DIC was selected to distinguish between models and select the most parsimonious model. When the best-fitting models included spatial autocorrelation, we extracted the range parameters to estimate the range of autocorrelation and ρ parameters to estimate correlation between seasonal fields. For the range of autocorrelation, we report the

distance at which spatial autocorrelation decayed to 0.5 (henceforth “halving range”; (Brooker *et al.* 2006)). Finally, we compared effect sizes from each model to investigate whether incorporating spatial autocorrelation altered any conclusions about the fixed effects. We particularly focussed on whether accounting for spatial autocorrelation altered the estimates for reproductive status effects, which have previously been demonstrated to impact both immunity and parasitism, and vary spatially across the population.

Results

Our models revealed strong and contrasting spatial trends in all but one of our response variables. All models but *E. cervi* were incrementally improved by first incorporating a spatial random effect and then by allowing it to vary between seasons (DIC values in Table 1; all secondary models had ΔDIC≥3.44). In all cases, including spatially distributed random effects improved model fit compared to fitting a subpopulation fixed effect (Table 1; ΔDIC≥2.4). The spatial fields of the random effects, taken from model sets 3-5, are displayed in Figure 2. For each response variable, we report the spatial field and results from both model set 3 (spatial field constant across the study period) and model set 4 (spatial seasons varying seasonally, with no correlation between fields). The exception is *F. hepatica*, for which allowing the seasonal fields to correlate in model set 5 improved model fit (ΔDIC=2.29, Table 1); therefore, for *F. hepatica,* we display the fields and results from model sets 3 and 5. Response variables differed considerably in terms of both their spatial fields (Figure 2) and the range at which they varied (Figure 3). Table 1 also displays the distance at which spatial autocorrelation reduced to 0.5 (“halving ranges”) and ρ values; as *E. cervi* models were never improved by the inclusion of the subpopulation fixed effect or by SPDE random effects (ΔDIC>1.36), we do not report these results further.

Strongyle nematode intensity exhibited weak spatial patterns, with a very short range of autocorrelation; this did not increase when spatial fields were allowed to vary seasonally (Figure 2-3, halving range<59.62M). Allowing the spatial field to vary between seasons resulted in similar patchy distributions which are hard to distinguish (Figure 2) but nevertheless improved model fit compared to all other models (Table 1, ΔDIC=4.25). *F. hepatica* demonstrated a strong spatial pattern, with high intensities in the mid- and south-valley decreasing to the north and northeast (Figure 2). This gradual, unidirectional trend was reflected in the long range of autocorrelation (Figure 3, halving range=1323M). Allowing the spatial field to vary between seasons improved *F. hepatica* model fit, but resulted in similar seasonal fields (Figure 2). This was reflected in the positive ρ parameter (ρ=0.67) derived from model 5, which was the best-fitting model for *F. hepatica*, demonstrating that seasonal spatial fields were substantially positively correlated.

When the spatial field was kept constant across the study period, total IgA and anti-Tc IgA both demonstrated a very short range of spatial autocorrelation (Figure 3, halving range<150.06M). Both antibody distributions were similar and negatively correlated with that of strongyles, being lower in the central north and higher in the south and edges of the study area (Figure 2). However, allowing both antibodies' spatial fields to vary between seasons improved model fit substantially compared to all other models (Table 1, ΔDIC<18.58), increased the range of autocorrelation (Figure 3, halving range>415.82M), and resulted in very different seasonal patterns (Figure 2). These patterns were similar between total IgA and anti-Tc IgA, although total IgA had a slightly larger range of autocorrelation (Figure 3, halving range=640.07M and 415.82M for total IgA and anti-Tc IgA respectively). The best-fitting model for total IgA and anti-Tc IgA was either model 4 or 5 for total IgA (Table 1, ΔDIC<2), while model 5 fit slightly better for anti-Tc IgA (ΔDIC=2.02). Hence model 4 is presented for total IgA as the model with fewer degrees of freedom, and model 5 is presented for anti-Tc IgA.

The subpopulation fixed effects broadly followed the spatial fields of the SPDE random effects (Figure SI1). Briefly, strongyles showed little difference across different regions, although estimates for the two northern regions (regions 3 and 5) did not overlap with zero when compared to the southern region 1. For *F. hepatica* intensities decreased moving northeast from region 1 to region 6, and all regions exhibited significantly decreased levels below the far south region 1. The reverse was true for *E. cervi* intensities. Patterns for total IgA and anti-Tc IgA are harder to interpret and less significant, but broadly the far south region 1 subpopulation featured higher antibody levels than northern regions (regions 2, 3, and 5 for total IgA and region 5 for anti-Tc IgA).

Most fixed effect estimates were only slightly modified by incorporating spatial autocorrelation structures in our models (Figure SI1). No estimates were reduced in significance except the seasonal effects in models 4 and 5 for *F. hepatica* and *E. cervi* (Figure SI1). Examining the spatial fields (Figure 2), this reduction in seasonal effect probably originated from competition between the seasonally varying spatial random effect and the season variable itself. Otherwise, effect estimates remained unchanged when spatial autocorrelation was included. This was particularly true for reproductive status effects, many of which actually increased slightly in magnitude when we accounted for spatial autocorrelation (Figure SI1). The models therefore replicated our previous study by demonstrating that reproductive investment was associated with lower antibody levels and higher strongyle intensities (Albery, Watt, et al. 2018).

Discussion

This study has revealed fine-scale spatial variation in immunity and parasitism at an individual level in a large wild mammal population. Spatial heterogeneity contributed considerably to between-individual differences in immunity and parasitism despite a total sampling area of only ~12.7 km^2. The scale of spatial dependence was therefore extremely short, and well within the scale of the study area. These findings are in accordance with a previous study demonstrating fine-scale immune variation in a discrete spatial context (within-site versus between-site) in wild mice (Abolins *et al.* 2018). We demonstrate similar spatial variation in a continuous context, and in both antibody levels and parasite counts, despite considerable mixing within the population. Furthermore, the response variables differed in terms of their spatial fields, the distances at which autocorrelation decayed in space, and their interactions with seasonality. Finally, spatial distributions of antibodies were not similar to any parasite distributions, implying that fine-scale environmental factors acting on exposure are more important than host immune susceptibility in driving spatial heterogeneity of parasite infection.

The scale of dependence and its importance for disease ecology studies

Understanding the spatiotemporal scale of disease processes is important for designing sampling regimes and disease control strategies (Caprarelli and Fletcher 2014; Lachish and Murray 2018). In this context, our results have several important general implications. Firstly, fine-scale trends like those exhibited here may scale up quickly where environments vary across larger distances, contributing to larger-scale geographic patterns of disease that are more commonly studied (Ostfeld *et al.* 2005; Murdock *et al.* 2017; Murray *et al.* 2018). Second, disease ecology and ecoimmunology studies that do not consider spatial autocorrelation, even over short distances, may be missing important sources of variation in immunity and exposure and risk reporting biased effect estimates. The persistent spatial trend seen in *F. hepatica* (Figure 2) demonstrates that different areas of a given study system can be consistently associated with either higher or lower parasitism, so that uneven sampling in space could introduce confounding variation and bias. In contrast, where the range of autocorrelation is extremely short, as in strongyles, sampling regimes that do not consider spatial dependence may incidentally sample areas of both high and low parasitism, reducing the risk of spatial biasing. The range of autocorrelation was well within the range of an individual deer's home range (Froy et al. 2018), implying that individuals may experience considerable variation in parasitism depending on their movement choices within this range. Trends are not necessarily similar across variables, complicating matters: most notably, spatial gradients of *F. hepatica* and strongyle intensity differed considerably both in range and patterns, and antibody hotspots did not align with parasite hotspots (Figure 2). Therefore, information on the spatial distribution of one immune or parasite measure could not be used to infer the distribution of another, and

appropriate sampling regimes will differ between response variables. Finally, all models except *E. cervi* were further improved when the spatial field was allowed to vary seasonally, and spatial patterns of antibody levels changed considerably between seasons (Figure 2). This confirmed our expectations that spatial fields would not be static in time (Hawkins 2012). Therefore, in some cases, even sampling from a wide, contiguous area may only capture a cross-sectional snapshot of the spatial dynamics of a given study system, necessitating longitudinal analysis.

Spatial heterogeneity has the potential to obscure or produce artefactual associations with other variables, modifying conclusions drawn from models without spatial dependence structures – in particular by inflating the type I error rate (Beale *et al.*, 2010; Pawley and McArdle, 2018). However, in this study, fixed effects remained largely unchanged when incorporating spatial dependence structures despite the importance of spatial heterogeneity (Figure SI1). In particular, previously reported reproductive status effects (Albery, Watt, *et al.* 2018) persisted or increased slightly in size, despite the fact that reproductive success varies across the study area (McLoughlin *et al.* 2006; Stopher *et al.* 2012). This demonstrates that spatial variation can contribute to ecological patterns of disease without necessarily obscuring other findings (Pawley and McArdle 2018). We suggest that disease ecology studies that examine wild populations attempt to investigate spatial variation to enrich their results, rather than viewing spatial autocorrelation as a nuisance (Pawley and McArdle 2018). In addition, although the spatial fields were broadly reflected by the subpopulation fixed effect results (Figure SI1), the spatial fields were more easily interpretable and increased model fit, and therefore incorporating spatial autocorrelation was advantageous. While integrating spatial dependence did not have severe impacts on effect sizes in our study, we lastly encourage researchers to consider accounting for spatial dependence even at the fine scales here to improve statistical inference and account for this variation.

Interpreting the spatial fields

The spatial fields derived from our models can help to indicate the factors determining immunity and parasite infection. Spatial trends of *F. hepatica* were especially stark, being much higher in the south of the study area and decreasing to the north and northeast (Figure 2). Given that the parasite distributions were not explained through differences in immune susceptibility, particularly considering minimal overlap with antibody level distributions (Figure 2), spatial patterns in parasite intensity likely instead resulted from spatial variation in exposure. This heterogeneity likely originated from the drier environment in the north compared to the wet, marshy ground in the south of the valley, the latter of which could be conducive to parasite persistence in the environment. After being excreted, *F. hepatica* eggs develop to form infectious miracidia, which seek out and infect *Galba truncatula* water snails

(Taylor *et al.* 2016; Beesley *et al.* 2018). After a period within the snail, cercariae are produced which encyst on vegetation as metacercariae to be consumed by deer. Wet areas are likely to host higher *G. truncatula* abundance, and warmer, wetter environments are conducive to fluke development and host seeking behaviour, both of which will produce higher exposure (Ollerenshaw and Smith 1969). The observed fluke distribution agrees with a number of studies in livestock demonstrating high fluke risk where grazing and wet areas intersect (e.g. Olsen *et al.*, 2015). Similar relationships with water sources are displayed by the human trematode *Schistosoma mansoni*, which shows a similar range of autocorrelation (Brooker *et al.* 2006). Our corroboration of these findings in a wild mammal implies that similar environmental risk factors may be influencing trematode infection in wild animals, humans, and livestock.

In contrast to *F. hepatica*, the spatial field of strongyle intensity is difficult to interpret: spatial autocorrelation introduced important variation, yet the range of autocorrelation was small, similar to that reported for human hookworm infection (Brooker *et al.* 2006), and displayed no discernible pattern either across the study period nor within seasons (Figure 2). Strongyles may be less impacted by environmental factors than is *F. hepatica* due to their direct life cycle, which does not involve a secondary host, such that spatial autocorrelation in intrinsic factors affecting susceptibility is more important than environmental effects on exposure and transmission. Host genetic similarity is a possible intrinsic factor producing the spatial autocorrelation seen in strongyle counts and antibody levels: both are heritable in ungulates (Bisset *et al.* 1992; Callaby *et al.* 2014; Hayward *et al.* 2014), and genetic relatedness is correlated with spatial distance in this system (Stopher *et al.* 2012). Alternatively, as social behaviours commonly covary with spatial behaviour (e.g. Sanchez and Hudgens 2015), the spatial patterns established here may be partially explicable through social metrics such as conspecific density. Future studies in this population could examine whether local population density and/or other social variables affect individuals' immunity and parasitism in ways that the INLA SPDE effect was unable to detect, potentially by using individual-level behavioural metrics derived from census data (Coulson et al. 1997; Froy et al. 2018).

Ecological and epidemiological implications

The fine-scale spatial heterogeneity demonstrated here has implications for the ecology and control of infectious disease in wild ungulate populations. For example, localised transmission hotspots may maintain parasite diversity, preventing competitive exclusion of parasites through geographic niche differentiation and contributing to the considerable genetic differentiation seen in liver fluke populations (Beesley *et al.* 2016). Additionally, when combined with sex-specific deer ranging patterns, spatial trends could contribute to previously observed sex biases in infection intensity (Albery, Kenyon, *et al.* 2018). Finally, it is possible

that the strong seasonality in ranging behaviour (Stopher et al. 2012) interacts with seasonal patterns of parasitism and immunity (Albery, Kenyon, et al. 2018). With more data, future investigations in this system may be able to examine these associations.

As *F. hepatica* is an important livestock parasite, fluke control initiatives should consider the presence of high-risk wet areas of grazing that may be used by deer populations. However, it is worth noting that the fluke hotspots here were observed at the per-capita count level, rather than as an absolute number of parasites in the environment. Given the higher deer density in the north, taking *F. hepatica* as an example, it is likely that the absolute number of fluke eggs being excreted in the north is higher than the south, but these parasites are less likely to complete their life cycle due to unsuitable environmental conditions. In the future, it may be possible to compare the excretion and movement patterns of the deer with pasture larval counts and snail sampling across the study area to examine the rate at which successful infection occurs, and to investigate whether deer living in the high-risk southern area of the valley may indeed be vectoring *F. hepatica* to the north (Chintoan-Uta *et al.* 2014; French *et al.* 2016).

Author contributions

GFA designed the study, collected samples, conducted labwork, analysed the data, and wrote the manuscript. DJB, FK, DHN, and JMP offered comments and suggestions on theory, analysis, and the manuscript throughout.

Acknowledgements

The authors thank the Division of Ecoimmunology and Disease Ecology, Division of Comparative Endocrinology, Division of Animal Behavior, and Division of Ecology and Evolution of the Society for Integrative and Comparative Biology as well as the Macroecology of Infectious Disease Research Coordination Network funded by the National Science Foundation (NSF DEB 1316223) for supporting the symposium “The scale of sickness: how immune variation across space and species affects infectious disease dynamics” financially. The long term red deer study is funded by the Natural Environment Research Council (grant number NE/L00688X/1), as is GFA’s PhD studentship through the E3 Doctoral Training Partnership (grant number NE/L002558/1). FK receives funding from the Scottish Government, RESAS, Strategic Research Programmes 2016-21. We thank Scottish Natural Heritage for permission to work on the Isle of Rum NNR and for the support of the reserve management team on the island. Thanks to Dave McBean and Gillian Mitchell at the Moredun Research Institute for their help with parasitological methods. The *Teladorsagia circumcincta* antigen was received from Moredun Research Institute, and was prepared by David Bartley, Alison Morrison, Leigh Andrews, David Frew and Tom McNeilly. Thanks also to Kathryn Watt

for assisting with immunological labwork and to Sean Morris, Alison Morris, Olly Gibb, and all other field assistants for their help in sample collection. Finally, we thank two anonymous reviewers for their helpful comments.

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