**Article:**
Rossi, Gianluigi, Crispell, Joseph, Brough, Tanis et al. (2022) Phylodynamic analysis of an emergent Mycobacterium bovis outbreak in an area with no previously known wildlife infections. Journal of Applied Ecology. pp. 210-222. ISSN: 0021-8901

https://doi.org/10.1111/1365-2664.14046

Received: 22 February 2021 | Accepted: 18 August 2021

DOI: 10.1111/1365-2664.14046

Journal of Applied Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

# Phylodynamic analysis of an emergent *Mycobacterium bovis* outbreak in an area with no previously known wildlife infections

Gianluigi Rossi[1] | Joseph Crispell[2] | Tanis Brough[3] | Samantha J. Lycett[1] | Piran C. L. White[4] | Adrian Allen[5] | Richard J. Ellis[6] | Stephen V. Gordon[2,7] | Roland Harwood[8] | Eleftheria Palkopoulou[9] | Eleanor L. Presho[5] | Robin Skuce[5] | Graham C. Smith[10] | Rowland R. Kao[1]

[1]Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, UK

[2]School of Veterinary Medicine, University College Dublin, Dublin, Ireland

[3]Advice Services Team, Service Delivery Directorate, APHA, Penrith, UK

[4]Department of Environment and Geography, University of York, York, UK

[5]Bacteriology Branch, Veterinary Sciences Division, Agri-food and Biosciences Institute, Belfast, UK

[6]Surveillance and Laboratory Services Department, APHA, Addlestone, UK

[7]Conway Institute, University College Dublin, Dublin, Ireland

[8]Veterinary Service, DAERA, Belfast, UK

[9]Department of Bacteriology, APHA, Addlestone, UK

[10]National Wildlife Management Centre, APHA, Addlestone, UK

**Correspondence**
Rowland R. Kao
Rowland.kao@ed.ac.uk

**Funding information**
Department for Environment, Food and Rural Affairs; Science Foundation Ireland, Grant/Award Number: 16/BBSRC/3390; Biotechnology and Biological Sciences Research Council, Grant/Award Number: BB/L010569/1, BB/L010569/2 and BB/P010598/1; Department of Agriculture, Environment and Rural Affairs, Northern Ireland Executive, Grant/Award Number: project 13-03-07

**Handling Editor:** Andrew Park

## Abstract

1. Understanding how emergent pathogens successfully establish themselves and persist in previously unaffected populations is a crucial problem in disease ecology, with important implications for disease management. In multi-host pathogen systems this problem is particularly difficult, as the importance of each host species to transmission is often poorly characterised, and the disease epidemiology is complex. Opportunities to observe and analyse such emergent scenarios are few.
2. Here, we exploit a unique dataset combining densely collected data on the epidemiological and evolutionary characteristics of an outbreak of *Mycobacterium bovis* (the causative agent of bovine tuberculosis, bTB) in a population of cattle and badgers in an area considered low risk for bTB, with no previous record of either persistent infection in cattle, or of any infection in wildlife. We analyse the outbreak dynamics using a combination of mathematical modelling, Bayesian evolutionary analyses and machine learning.
3. Comparison to *M. bovis* whole-genome sequences from Northern Ireland confirmed this to be a pathogen single introduction from the latter region, with evolutionary analysis supporting an introduction directly into the local cattle population 6 years prior to its first discovery in badgers.
4. Once introduced, the evidence supports *M. bovis* epidemiological dynamics passing through two phases, the first dominated by cattle-to-cattle transmission before becoming established in the local badger population.
5. *Synthesis and applications.* The *Mycobacterium bovis* emergent outbreak that was the object of this study was of considerable concern because of the geographical distance from previously known high-risk areas. Initial decisions about the outbreak control were supported by the whole-genome sequencing data. The further analyses described here were used to estimate the time of introduction (and

therefore the likely magnitude of any hidden outbreak) and the rates of cross-species transmission, and provided valuable confirmation that the extent and focus of the imposed controls were appropriate. Not only do these findings strengthen the call for genomic surveillance, but they also pave the path for future outbreaks control, providing insights for more rapid and decisive evidence-based decision-making. As the methods we used and developed are agnostic to the disease itself, they are also valuable for other slowly transmitting pathogens.

**KEYWORDS**

bovine tuberculosis, emerging infectious disease, inter-species transmission, pathogen establishment, phylodynamic, surveillance, whole-genome sequencing, wildlife–livestock interface

## 1 | INTRODUCTION

Pathogens able to spread at the interfaces between livestock, wildlife and humans are one of the most serious threats to human health, wildlife conservation and livestock economic sustainability (Gortazar et al., 2015; Wiethoelter et al., 2015). Generally, the spread of a pathogen is enhanced when it co-circulates in multiple sympatric host species, as interspecific and intraspecific transmissions can complement each other, resulting in pathogen persistence (Craft et al., 2008; Haydon et al., 2002).

*Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex (MTBC; Smith et al., 2006), is responsible for bovine (or animal) tuberculosis (bTB) in domestic cattle and a range of wild mammals, including European badgers and deer in Britain and Ireland (Crispell et al., 2020; Godfray et al., 2013; Skuce et al., 2020), deer and wild boar in the Iberian Peninsula (Anderson et al., 2013), deer and elk in Michigan, US (Salvador et al., 2019), possums in New Zealand (Anderson, Ramsey, et al., 2013; Crispell et al., 2017) and water buffalo in South Africa (Fitzgerald & Kaneene, 2013).

In Britain and Ireland several studies have established an association between the presence of infected badger populations and the persistence of bTB in cattle (Donnelly et al., 2003, 2006; Martin et al., 1997). More recently, researchers have demonstrated that the same *M. bovis* strains are co-circulating in domestic cattle and sympatric badgers in endemic areas, first using pathogen's DNA genotyping techniques (Olea-Popelka et al., 2005; Woodroffe et al., 2009), and later using whole-genome sequencing (WGS; Biek et al., 2012; Crispell et al., 2019). Despite the UK government's efforts to control and eradicate bTB, the last decades have seen an increase in the number of cases and substantial expansion of bTB endemic areas, in particular in England and Wales (Brooks-Pollock et al., 2014; Smith et al., 2006). The eradication efforts for this disease cost the UK government around 100 million pounds per year in England alone (Defra, 2011; Godfray et al., 2013).

Collecting reliable and up-to-date data about wildlife populations can be challenging at broader scales (Craft, 2015), with badger populations in most regions of Britain and Ireland making no exception. Although there have been a large number of studies characterising badger populations dynamics and behaviour [see Roper (2010) and references therein], only few populations such as at Woodchester Park (Gloucestershire, England) have been subject to long-term sampling (Delahay et al., 2013). Furthermore, surveys across England have shown that, since the mid-1980s, the estimated number of badger social groups has grown by 2.6% annually, increasing the uncertainty around badgers' potential to become a bTB reservoir in different regions (Judge et al., 2014). The scarcity of detailed information on badgers over large areas of the country may prevent the design of effective disease control practices when bTB is introduced.

Further complications arise from the difficulty of estimating the true prevalence of *M. bovis* infection in both badgers and domestic cattle. While *M. bovis* is characterised by slow replication with the potential for latent periods of variable length within the host (Cassidy, 2006; Pollock & Neill, 2002), the accuracy of currently available diagnostic tests is suboptimal in both cattle (Nuñez-Garcia et al., 2018) and badgers (Drewe et al., 2010). All these factors contribute to obscure the disease dynamics at the local scale, and prevent a clear understanding of the relative roles of the two species in bTB maintenance and spread, which in turn is hampering the disease control and the effectiveness of disease surveillance strategies.

Within the English regions designated as low-risk areas (LRAs), the eastern part of the county of Cumbria in north-west England ('East Cumbria' hereafter) has recently experienced a bTB outbreak of unusual magnitude and duration for this area. The outbreak began in 2014 and by mid-2019, through enhanced TB surveillance testing of all cattle herds in the affected area, 39 breakdowns (positive cattle herds) across 33 premises (Defra, 2019a) had been detected. The outbreak was caused by a strain (genotype) of *M. bovis* uncommon in England, but well-established in Northern Ireland, which preliminary molecular analyses highlighted as the likely area of origin. The same strain of *M. bovis* was subsequently found in the local badger population, at first within the 'found-dead' surveillance initiated by the Animal and Plant Health Agency (APHA) in September 2016 (Defra, 2019a). As a consequence of this epidemiological link and following a public consultation, a culling licence was issued in the outbreak area in Autumn 2018, which confirmed the establishment of this *M. bovis* genotype in the local badger population.

This study aimed to shed light on the dynamic spread of *M. bovis* when introduced in a two-host system in a non-endemic area, using the outbreak in East Cumbria as a case study. We describe the East Cumbria outbreak spatial and temporal characteristics, identify the factors which led to *M. bovis* infection becoming established in a wildlife population, and estimate the number of intra-species and inter-species transmission events. Our approach includes the use of forensic molecular epidemiology (Kao et al., 2014), thanks to the availability of over 60 isolates of *M. bovis* from the outbreak with usable sequences, complete with precise metadata including dates, locations and, for cattle, animal and farm identifications.

Results of this study are an important step towards a deeper understanding of bTB introduction and establishment into non-endemic areas, thus providing crucial insights for bTB management and highlighting the importance of tailored control strategies in non-endemic areas.

## 2 | MATERIALS AND METHODS

### 2.1 | Outbreak history

In November 2014, typical bTB lesions were detected via routine slaughterhouse inspection of a 7-month-old male calf from a dairy herd in East Cumbria. Bacteriological culture of the lesions yielded an unusual genotype of *M. bovis*, designated 17:z by APHA (Figure 1a). Following this first report, 23 more cattle were confirmed to be infected with the same strain in East Cumbria, with the last of these detected in November 2018. Further cattle were declared bTB positive (using the tuberculin skin test and/or supplementary interferon-gamma blood tests) in the outbreak area, although *M. bovis* bacilli could not be isolated. The 24 cattle infected with *M. bovis* 17:z genotype included three animals detected in Cumbria but outside the outbreak area, and three in the neighbouring counties of Lancashire (two) and Yorkshire (one), all deemed likely to be part of the same outbreak due to associations through contact tracing.

During 2016 and 2017, respectively, two and 35 roadkill badger carcasses were reported to the local authorities within the designated outbreak area. Three carcasses, retrieved respectively in January, February and April 2017, were positive for *M. bovis* on culture (while two carcasses were unsuitable for inspection, Figure S1.1), and all three positive animals were infected with the 17:z genotype.

Culling operations from September to November 2018 resulted in 602 culled badgers, of which 369 were submitted for post-mortem inspection and laboratory testing (Defra, 2019a). In total, 42 were culture positive for *M. bovis*, with 38 isolates yielding a whole-genome sequence of sufficient quality to enable phylogenetic analysis (Defra, 2019b).

Data on found-dead surveillance in 2018 and 2019, but prior to the second culling season, included an additional 42 retrieved carcasses (29 in 2018, and 13 in 2019), 24 of which were negative for *M. bovis*, 15 were unsuitable for post-mortem inspection, and three still pending a bacterial culture. This study includes all the available data collected to November 2019.

### 2.2 | Data

#### 2.2.1 | *M. bovis* isolates and metadata

Test positive cattle, found-dead and most culled badgers were subject to post-mortem and culture of suitable tissues at the Animal and Plant Health Agency (APHA), with positive cultures subjected to genotyping and WGS at the Central Sequencing Unit in Weybridge. Sixty-five *M. bovis* sequences were available from East Cumbria (24 from cattle, 3 from roadkill badgers and 38 from culled badgers). The sampling timeline of the available sequences is reported in Appendix S1, Figure S1.1. The metadata included a unique identifier, the sampling date, location coordinates (for badger isolates) and the farm's county-parish-holding (CPH) code (for cattle sequences only). The isolates and raw sequence data were processed using the same pipeline as described by Crispell et al. (2019).

#### 2.2.2 | Badger population

A total of 160 badger setts were identified in and around the outbreak area in 2017/2018 by the APHA, with 117 of them in the 2018 culling permit area. Badgers were both shot and trapped, with trapped animals subjected to post-mortem analysis, and population data (number of badgers removed, TB positive, negative and not determined) were available for 99 setts.

#### 2.2.3 | Cattle population and outbreak area definition

To obtain all infected cattle life histories (movements, birth and death) we first matched the sequences' unique identifier in the SAM dataset, then we extracted the data from the Cattle Tracing System (CTS) using the animal unique identifier.

The outbreak area was defined as the area within the minimum circle around the sequences' sampling locations and all 160 badger setts, plus all the parcels assigned to the infected farms which are contiguous to the above described circle (Figure 1a). This study included all premises active between 1 January 2010 and 31 December 2018 (which reported any cattle movements, births or deaths) directly located in the area or which owned a parcel in the outbreak area. A total of 336 cattle premises was selected.

#### 2.2.4 | Northern Ireland TVR data

The Test, Vaccinate or Remove (TVR) trial in Northern Ireland ran from 2014 to 2018, and it was designed to determine whether a combination of vaccination and field test could be effective in controlling *M. bovis* infection in badger populations (DAERA-NI, 2015). During this period *M. bovis* samples were taken from infected cattle and badgers in the area for culturing.

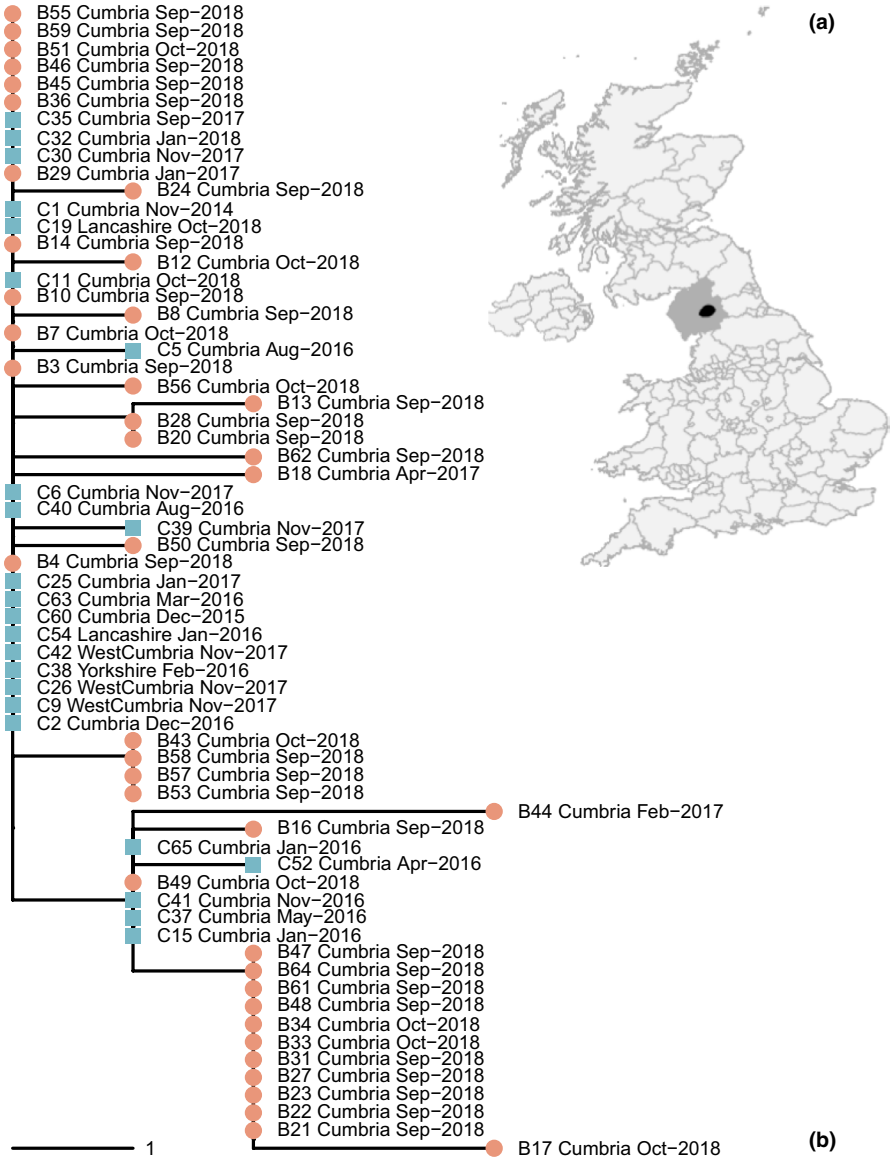


**FIGURE 1** East Cumbria outbreak area and phylogenetic tree. (a) East Cumbria outbreak area (black) location in Cumbria (dark grey). The exact locations of cattle premises and badgers' sett cannot be disclosed due to privacy reasons. (b) All Cumbria outbreak *Mycobacterium bovis* genomes phylogenetic tree (distance calculated in single-nucleotide variants, SNVs, and the tree was computed with a neighbour joining algorithm). Red dots represent badgers, and blue square cattle, and the label report the code assigned to each individual, sampling location and sample date

All positive cultures were sequenced at the Agri-Food and Biosciences Institute in Belfast (AFBI-NI). Moreover, archived *M. bovis* isolates stored as part of routine surveillance operations in the TVR area prior to the start of the trial were selected for sequencing. These additional isolates were sourced from routine test and slaughter surveillance of cattle or roadkill badgers.

From the TVR area in Northern Ireland, 544 *M. bovis* genomes were sourced from infected cattle (479) and badgers (65), sampled from 1996 to 2017 (Akhmetova et al., 2021).

### 2.2.5 | Epidemiological factor analysis

We investigated the effect of population, temporal, spatial and contact network covariates in the available *M. bovis* genomic data (Crispell et al., 2019). Specifically, we used these factors as explanatory variables in a regression model, where the single-nucleotide variant (SNV) distance between hosts' sequences was the dependent variable. For this analysis, we used a Boosted Regression Tree (BRT) model (Elith et al., 2008), fully reported in the Appendix S1 (Section 2).

## 2.3 | Pairwise KFEs and transmission tree

We calculated the transmission probability between each pair of animals with *M. bovis* sequences using the Kolmogorov forward equations (KFEs; Rossi et al., 2020). The KFEs consist of a set of ordinary differential equations tracking the probability of a system to be in a given state through time (Keeling & Ross, 2008; Sharkey, 2008). In the pairwise transmission case, the system was given by the combination of the two hosts' disease progression state and by the number of SNVs on its *M. bovis* strain. We assumed that, at the time of infection, the two pathogen strains found in the source and recipient

hosts are identical. After the infection, the two strains start to replicate, and thus substitution on the pathogen DNA happens at a rate $\mu$ (*substitution rate*), generating SNVs. Because the two strains are diverging, we will call the SNVs found in a strain sampled in the source host A [recipient host B] as *divergent* SNVs, or *divA*[*divB*]. The sum of *divA* and *divB* is the SNV distance between the strains.

To use this methodology, we need three main pieces of information: the pathogens' sequences, the two hosts' sampling times and an underlying model representing the disease progression. For bTB progression, we used a simple Susceptible-Exposed-Infectious model (Brooks-Pollock et al., 2014; Rossi et al., 2015), where a susceptible individual can become exposed (latently infected) after a transmission from a host at *infection rate* $\beta$, and exposed hosts move to the infectious state with *transition rate* $\sigma$ (or after a latency period of average $1/\sigma$). In this study, we also considered the birth date of the cattle, in order to limit the time span where each cattle could have been infected first. For badgers we assumed a constant death rate, based on the observation that less than 0.1% of individuals would survive past 8 years of age (Roper, 2010). The epidemiological parameters were chosen according the most recent literature (see Rossi et al., 2020), and in order to account for their variability, for each pairwise transmission we tested 10,000 combinations of randomly selected parameters combinations and chose the one returning the highest probability.

As in a previous analysis by Rossi et al. (2020), we assembled the most likely transmission tree by progressively selecting the pairs with the highest transmission probability, and excluding those not possible given the previously selected ones avoiding both loops (e.g. if A → B and B → C were selected, B → A, C → B and C → A were excluded) and time inconsistencies (e.g. if A → B was selected and C was removed before the transmission, B → C was excluded). Finally, we computed 10,000 'random trees' built by selecting random transmission pairs (except the ones for which the probability was zero because the cattle's life spans did not overlap) to compare with the most likely tree as computed by our ordered method.

## 2.4 | BASTA analysis

As phylogenetic evidence suggests a large amount of inter-species transmission is occurring in East Cumbria, the next critical question is '*in what direction*?'. We used the Bayesian Structured coalescent Approximation (BASTA) package (De Maio et al., 2015) with the Bayesian evolutionary analysis platform BEAST2 (Bayesian Evolutionary analysis by Sampling Trees; Bouckaert et al., 2014) to estimate *M. bovis* inter-species transmission rates. The BASTA package estimated these rates while accounting for the known structure and sampling biases in the study population. In the current study, the sampled *M. bovis* population was split into four different subpopulations based on host species (badger or cow) and location (Cumbria or TVR, Figure S4.1). Importantly, it is assumed that transmission from TVR to Cumbria only occurred in one direction, as the Cumbria clade is monophyletic within the larger TVR phylogeny (Figure 2).

Evolutionary analyses using BASTA require the presence of a temporal signal in the *M. bovis* genomic data. With a temporal signal, the accumulation of substitutions will be tied to the evolutionary processes of the sampled population, making it possible to leverage genetic variation to estimate evolutionary dynamics such as between-species transition rates. A root-to-tip versus sampling time regression was used to determine whether a measurable temporal signal was present in the *M. bovis* genomic data (Figure S4.2). The positive trend observed in this regression indicates the presence of a weak temporal signal, which allowed us to proceed with the evolutionary analyses in BASTA. Due to computational complexity, the 544 *M. bovis* genomes available within the outbreak clade (orange clade, Figure 2) had to be subsampled. First, it was only from 2014 to 2017 that the sampling of cattle and badgers in the TVR area could be considered approximately equal (in terms of effort). Therefore, only genomes sourced from infected cattle and badgers sampled from 2014 to 2018 were included in the BASTA analyses. The window was extended to 2018 to include the genomes sourced from Cumbria. The subsampling was then weighted to include samples from as many years as possible and from equal numbers of cattle and badgers. The subsampling was conducted 10 times, each time selecting 20 badgers and 20 cattle-derived *M. bovis* genomes from Cumbria and 40 badger and 40 cattle-derived genomes from the TVR area. A root-to-tip versus sampling time regression was conducted for each subsample and found to be positive in all cases. Each subsample was then analysed separately in BASTA. The subsampling, together with the provided population structure, allowed to reduce the temporal sampling biases in the BASTA analysis (Crispell et al., 2019).

# 3 | RESULTS

## 3.1 | Outbreak phylogeny and Northern Ireland isolates

The phylodynamic tree of the East Cumbria outbreak is reported in Figure 1b. Early analyses identified a genotype usually found in Northern Ireland; further evidence showed the existence of cattle movements from this area to England and Wales. Coincidentally, the origin area included the recently completed TVR trial area in Northern Ireland where extensive *M. bovis* WGS had already been done—these isolates were included in our analyses.

The complete phylogenetic tree (Figure 2) confirmed the association between the *M. bovis* circulating in the Northern Irish TVR area and in East Cumbria; thus, it appeared that the East Cumbrian outbreak likely originated from the dominant strain circulating in or around the TVR area (Figure 2, orange branches) via movement of infected cattle, although the first introduction was not identified.

## 3.2 | Epidemiological investigation

The index animal in this outbreak was a homebred calf that had never left its birth farm until it was moved to slaughter.

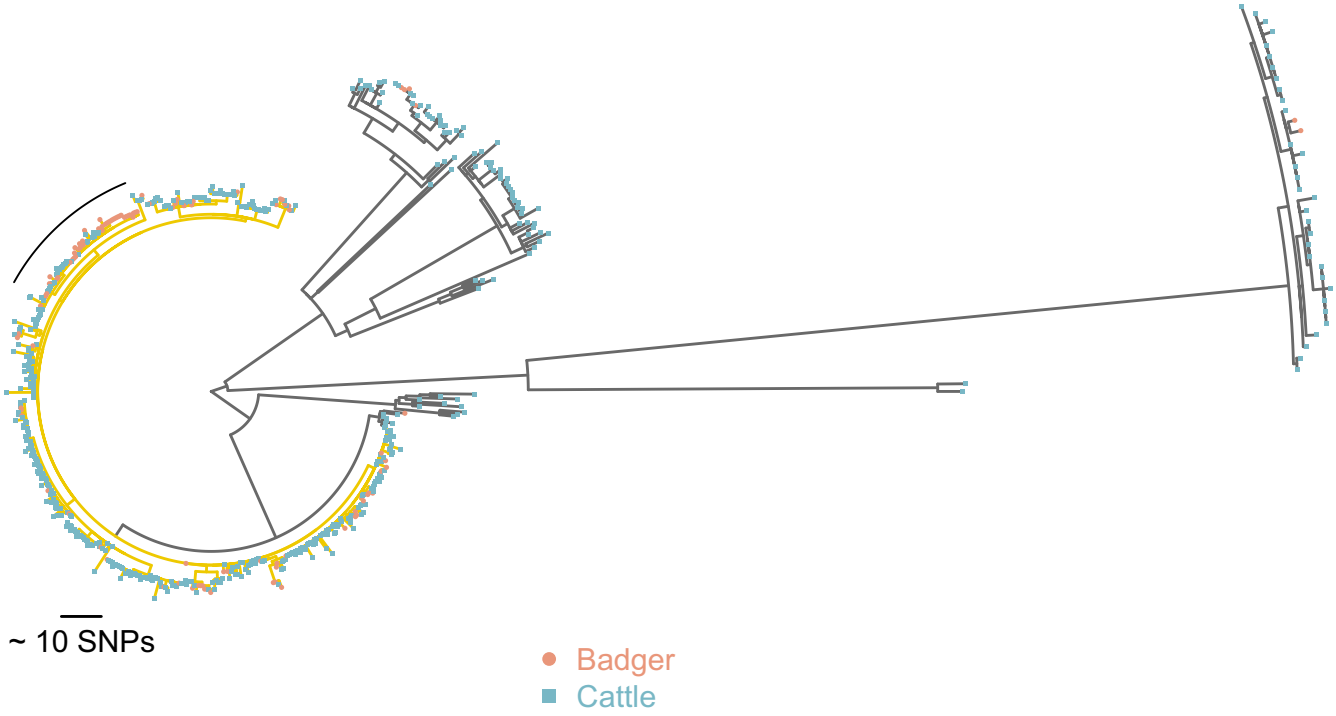


**FIGURE 2** East Cumbria and TVR *Mycobacterium bovis* phylogenies. A maximum likelihood phylogeny of the 611 *M. bovis* genomes combining both Cumbria and the Test, Vaccinate or Release (TVR) area in Northern Ireland populations. The tree is rooted with the *M. bovis* reference genome AF2122/97. The *M. bovis* genomes sourced from infected cattle and badgers in Cumbria are highlighted with a black semi-circle at the top left. The branches of the clade containing the *M. bovis* genomes sourced from Cumbria, and those from the TVR area that are most similar is highlighted in yellow. The two clades on the right show lineages circulating in the TVR area but distinct from the 17:z one

Therefore, this animal could not have been the 'case zero'. We attempted to identify the first infected individual introduced in the area by analysing all the Cattle Trace System dataset records that included animals born in Northern Ireland or in the Republic of Ireland from 2009 to 2014. Tracing back the direct movements from Northern Ireland to the outbreak area indicated a limited number of 'first arrival' premises (on average 9.3 per year, range 5–15), but unfortunately an obvious first introduction did not emerge. Conversely, searching for indirect links between Northern Irish farms by selecting other British farms with links to the Cumbrian outbreak which previously imported animals from Northern Ireland, provided too many potential 'arrival' premises (on average 216.5 and range 165–247, farms in the outbreak area per year).

### 3.3 | Pairwise transmission probability and most likely transmission tree

The pairwise transmissions probability matrix calculated using the KFEs (Rossi et al., 2020) is reported in Figure 3. The index animal (C1) infection source might have escaped detection, since the transmission probabilities from other detected animals are low (median $0.13 \times 10^{-3}$, range $0–1.98 \times 10^{-3}$). Similarly, two of three initially reported roadkill badgers (B44 and B18) had, respectively, the lowest and the third lowest average and maximum transmission probability from other detected animals (Figure 4). This indicates that animals infected early in the outbreak likely escaped detection (either the 4-year herd testing or carcass inspection), in particular the 'case zero' (i.e. the first cow imported infected with the 17:z genotype of *M. bovis*).

In general, within-species transmission probabilities were higher than between-species ones (Figure S3.1), and the phylogenetic root-to-tip temporal signal was strong ($R^2 = 0.39$, $p$-value ~0; see Figure S4.2). This was consistent with the BRT model results, where the temporal signal was identified as the most important predictor of the SNV distance.

Results showed that random trees had a median [95th CI] of 11[6–16] cattle-to-cattle, 21[15–26] badger-to-badger, 20[14–25] cattle-to-badger and 13[7–18] badger-to-cattle transmissions (Figure S3.3). The most likely transmission tree (Figure 5) showed that most of the transmissions in this system likely happened within species, that is, 20 cattle-to-cattle and 29 badger-to-badger transmissions respectively. Inter-species transmissions most likely included 12 cattle-to-badger transmissions, and three badger-to-cattle transmissions. When comparing these results with the randomly computed trees, the most likely tree showed a lower number of cross-species transmissions and a higher number of within-species transmissions, with the estimates not included in the 95th CI of the random trees ones.

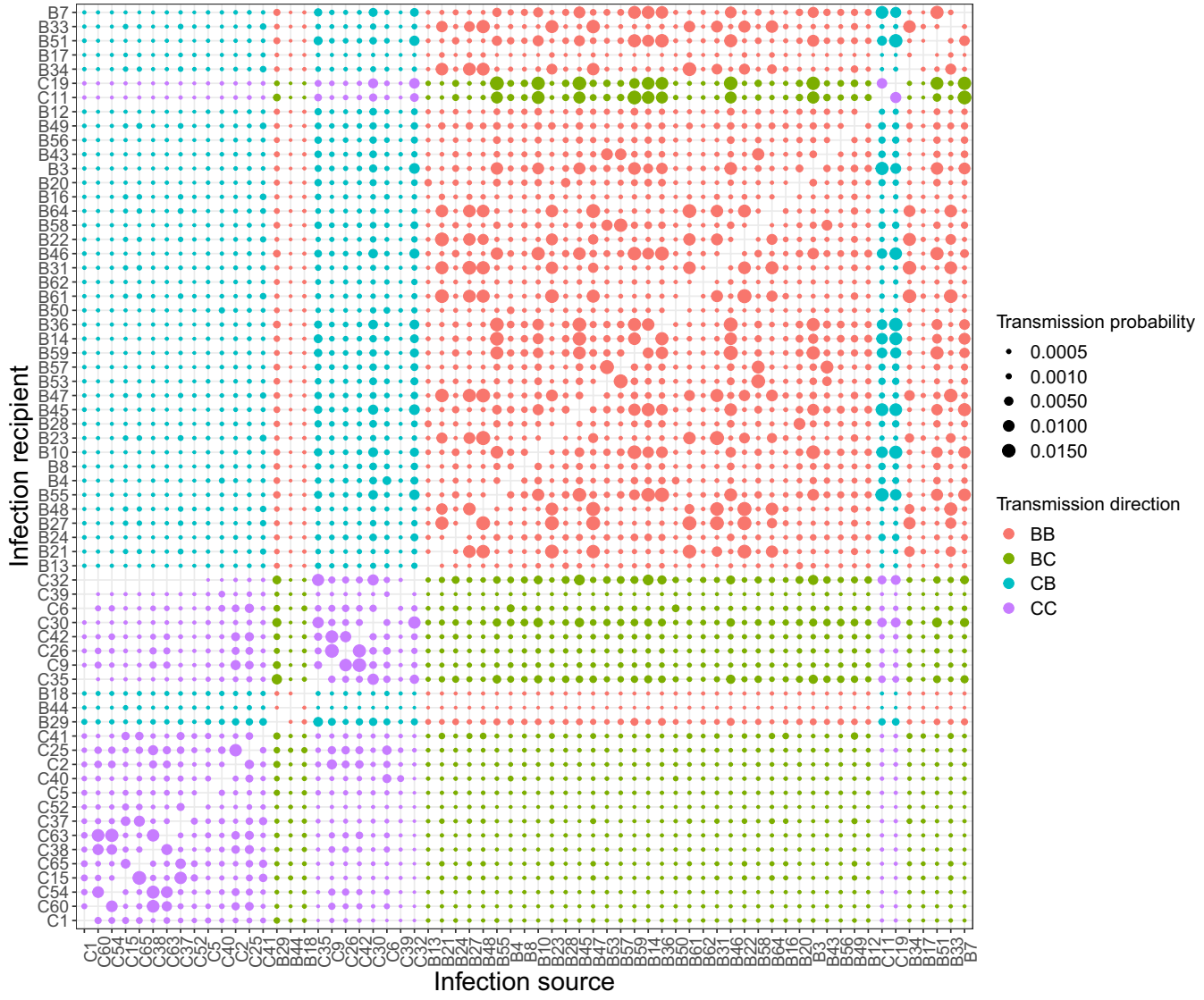


**FIGURE 3** East Cumbria outbreak transmission matrix. Pairwise transmission probabilities between infected animals in the East Cumbria outbreak. Animals are reported in *x* (source animal) and *y* (infected animal) axes in the order they have been sampled, and they are labelled from one to 65 and the species name (B for badgers and C for Cattle). Different colours correspond to transmission directions (red: badger-to-badger, green: badger-to-cow, light blue: cattle-to-badger and magenta: cattle-to-cattle)

### 3.4 | BASTA analysis

The transmission rates estimated by BASTA on the 10 subsamples suggest that during this outbreak, cattle-to-badger transmission occurred much more frequently (at least an order of magnitude) than badger-to-cattle one (Figure 6). Moreover, there is little support for the inclusion of badger-to-cattle transmission in the structured population model. In contrast, there is strong support for transmission of *M. bovis* from the sampled Northern Ireland area into the Cumbria area via cattle. Figure S4.3 shows the rate estimates produced using sampling dates but no genomic data. Given the contrasting rates shown in Figure 6 and Figure S4.3, there was strong evidence of a sufficient signal in the *M. bovis* genomic data to estimate the transmission rates. Lastly, good agreement across the 10 subsamples suggests that the estimated rates were robust to any inter-subsample variation.

Analyses in BASTA leveraging the temporal signal in the *M. bovis* genomic data were used to estimate the timing of *M. bovis* transmission from cattle in the TVR area to cattle in Cumbria (Figure S4.4). While credible intervals around these estimates were very broad, the transmission event was estimated to have occurred in March 2011 (lower 2.5% bound estimate: August 2001; upper 97.5% bound estimate: April 2014). This reflects the slow and variable replication rate characteristic of *M. bovis*.

## 4 | DISCUSSION

While cattle movements are known to be responsible for bTB transmission over long distances (Brooks-Pollock et al., 2014; Gilbert et al., 2005; Green et al., 2008), uncertainty remains around the role

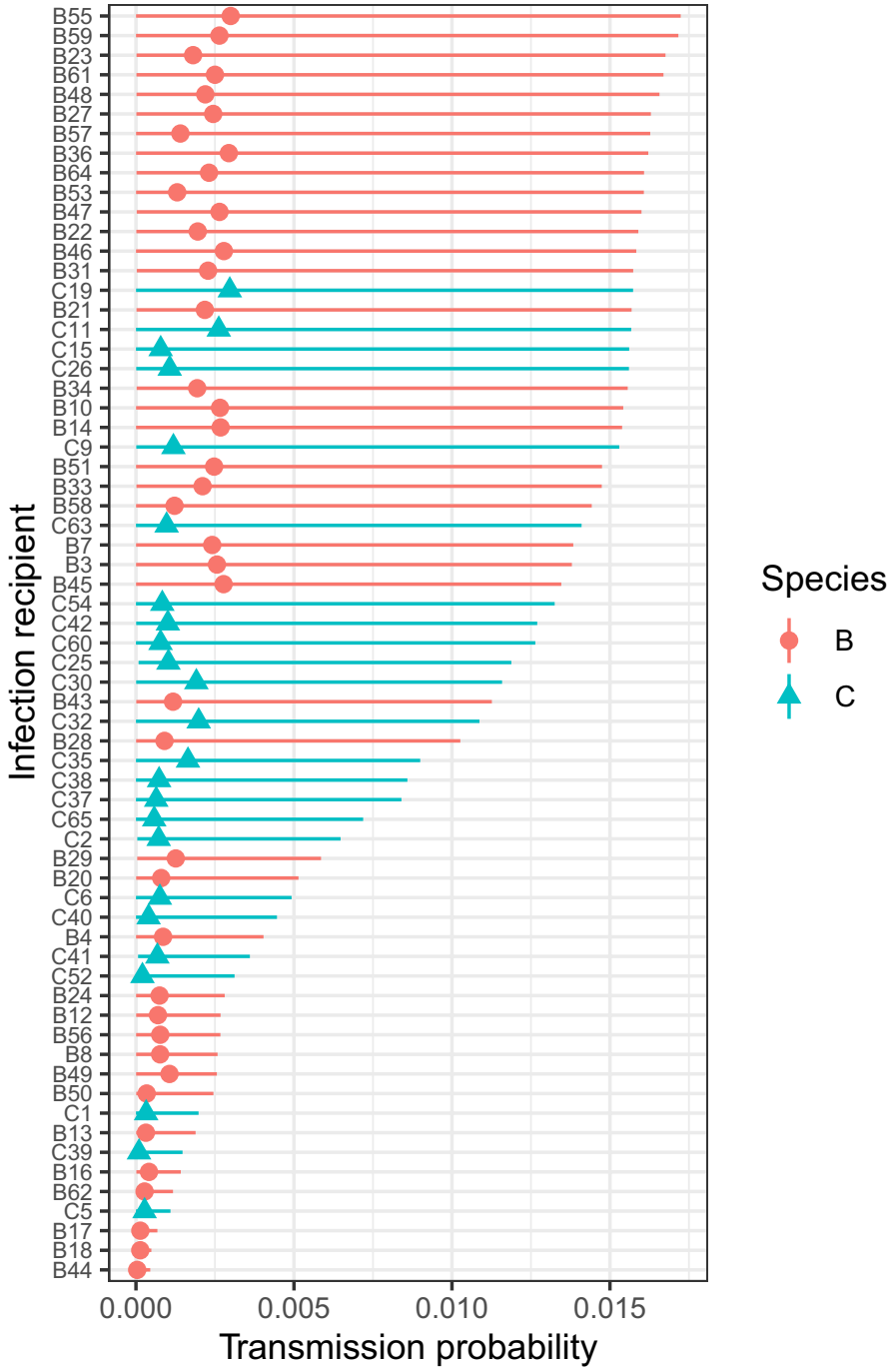


**FIGURE 4** East Cumbria outbreak transmission probability to detected infected animals. Average (dots/triangles) and range (line) of transmission probabilities (*x* axis) across all potential infection sources to each infected animal (*y* axis). Red lines/dots correspond to badgers and blue lines/triangles to cattle

of badgers in maintaining and spreading *M. bovis* infection at local scales, complicating the formulation and execution of control policies. Here, WGS proved crucial by indicating high genetic similarity between sequences found in Northern Ireland and Cumbria, and among all sequences found in the latter. This pointed to a recent, single introduction, supporting a policy of more intense controls in a smaller area. If the genetic similarity had been lower, more intensive disease management over a broader area would likely have been necessary. The evidence of transmission from badgers-to-cattle also supported the necessity of badger controls for cattle disease management. This is a crucial consideration given the extensive controversies regarding badger controls in the United Kingdom.

Our results also highlight the role of disease management in cattle in preventing endemic problems; after an initial seeding of *M. bovis* into the local East Cumbria cattle population, it took some time and a large outbreak in the cattle population before the infection became established in local badgers, but with evidence that subsequent rapid circulation in badgers lead to the observed 11% badger prevalence in the autumn of 2018, with peaks up to 21% in the core area (Defra, 2019b). Whether this is because of limited opportunities for cattle-to-badger transmissions, or limited opportunities for badger-to-badger ones (e.g. only a few badger setts might have sufficient densities to allow sustained *M. bovis* circulation), is unclear.

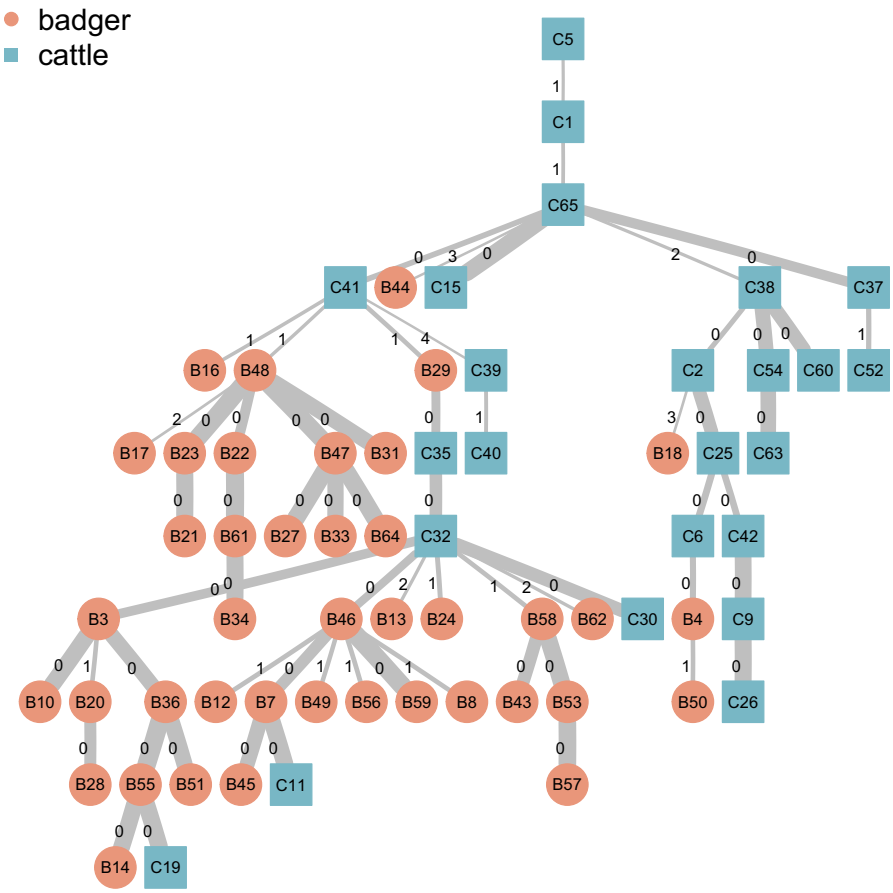


FIGURE 5 East Cumbria outbreak likely transmission tree. Most likely transmissions considering all the detected *Mycobacterium bovis* infected individuals in the East Cumbria outbreak (transmissions from top to bottom). Red circles correspond to badgers, and blue squares to cattle. Edge thickness is proportional to the pair transmission probability and the edge label (dark green) indicates the single-nucleotide variant (SNV) distance between the two individuals

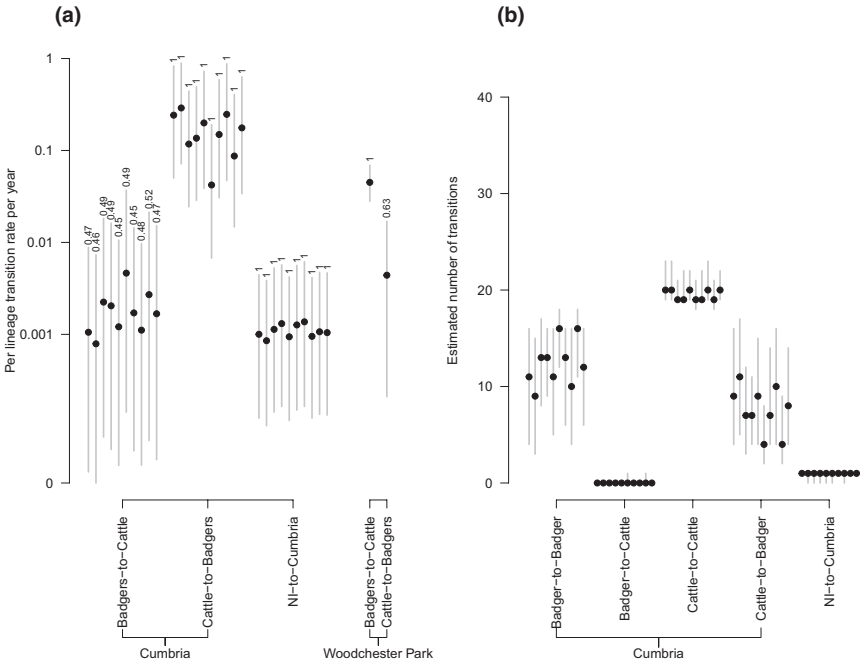


FIGURE 6 Transmission rates and number of transmissions estimated with BASTA. (a) Inter-species and Northern Ireland to Cumbria *Mycobacterium bovis* transmission rates (*y* axes, log scale) estimated using BASTA, based on analyses that used the genomic data and compared with the results for Woodchester Park (Crispell et al., 2019). Dots[bars] represent the posterior distribution median[95th CI], while the value plotted above each line represents the estimated posterior probability that the transition rate existed. (b) Number of transmission events estimates (*y* axes) between cattle and badgers, median[95th CI] showed by dots[bars]

A concern might be that transmission tree outcomes have been affected by the sampling timeline, since early in the outbreak there are fewer *M. bovis* available sequences, mostly coming from cattle and with no sampling effort in the badger population. However, the BASTA analysis accounts for the impact of unbalanced sampling (De Maio et al., 2015), and our conclusions are also supported by a low genetic diversity in the recovered badger *M. bovis* isolates, which points to a relatively recent outbreak in the badger population. The

weak probabilities associated with the most likely links in the earlier stages of the outbreak suggest the existence of missing transmission links. While we cannot discount the possibility that badgers were involved, the relatively low diversity of the sequences found later is consistent with no badger early involvement and, therefore, with no early bTB establishment in the local badger population.

Similar to a previous study (Crispell et al., 2019), the BRT analysis (Appendix S1, Section 2) indicated temporal bacterial isolation differences, and spatial distance between hosts, as the most important factors predicting inter-*M. bovis* genetic distance. While contacts considering single species only (animal movements for cattle and sett adjacency for badgers) were not significant in any models, fine-scale spatial effects captured by the spatial network model proved to be relevant. Complex interactions between the two species might not be clear when considered individually, but become evident when both species contact networks are included in a multi-layer network (Kinsley et al., 2020; Silk et al., 2018).

In the present study system, the BRT model poorly explained the *M. bovis* genetic distance in cattle, despite more data availability with respect to badgers. This and the good explanatory power of covariates related to the land parcels spatial network hint at potentially hidden contact patterns in the cattle dataset. While the inability to isolate *M. bovis* in all herd breakdowns or in all cattle is a possible explanation, unrecorded movements, such as cattle grazing in several land parcels belonging to the same farm but not contiguous to the farm (Campbell et al., 2019), could also be a cause. In general, landscape and farmland fragmentation and spatial distribution, ignored when considering farms' main building locations only, have the potential to complicate disease investigations. This calls for explicitly considering the spatial landscape and for management to be adapted to specific contexts defined by detailed veterinary investigation.

An important observation is the lesser role of badgers in the local persistence of the disease, contrary to what observed in a bTB endemic area (Crispell et al., 2019). Differences in the wildlife and cattle demographics may be important, as might the 'age' of the outbreak, as a build-up in badger infections may be required before badger-to-cattle infections become probable. While in both areas breaking the transmission at the wildlife/livestock interface is likely critical, the rapid decline in East Cumbria cattle bTB shows that there are substantial advantages in the early response to outbreaks within LRAs (non-endemic) to prevent bTB establishment. This was supported by a substantial reduction in badgers prevalence during the 2019 culling campaign (Defra, 2020b) and zero infected badgers found in the 2020 campaign (Defra, 2021). Questions remain whether East Cumbria outbreak could have generated inter-species dynamics similar to the ones observed in South-west England, if left unmanaged. The answer is likely to be in part determined by the different landscape differences and rearing practices, which may affect the *M. bovis* dynamic spread.

The first introduced infected bovid likely escaped detection at slaughter, highlighting the limitations of surveillance strategies in LRAs and the potential for good biosecurity to reduce the risk of onward transmission to wildlife even in these areas. This also led to policy changes: first, in April 2016 Defra adopted mandatory post-movement bTB testing of all cattle moved from high-risk to LRAs (Defra, 2016); second, Defra organised a steering group involving local stakeholders to provide advice to farmers and further improve biosecurity (Defra, 2020a). Finally, for the third year of control the non-core outbreak's area was moved from cull to ring vaccination (Defra, 2020a), which is a change from the other badger management taking place in the endemic bTB area.

For low-diversity pathogens such as *M. bovis*, genomic data alone provide limited understanding of an outbreak (Campbell et al., 2018). Here, we combined these with detailed epidemiological information to forensically disentangle a bTB outbreak in a previously naïve area, and gather crucial insights on the roles of wildlife and domesticated livestock. We highlight how local spatial dynamics might affect pathogen spread during the early phases of an outbreak, with early spread in cattle ultimately leading to establishment in the badger population. While we found little evidence for substantial badger-to-cattle transmission, previous analyses (Crispell et al., 2019) showed that under suitable conditions badgers can be important contributors to cattle disease.

In conclusion, this work highlights that disease management strategies in endemic and non-endemic areas should be considered independently. Our results showed how genomic surveillance is crucial for both first assessment and outbreak monitoring purposes, and how this strongly supports adaptive management approaches informed by the outbreak evolving dynamics.

## ACKNOWLEDGEMENTS

This work was supported by BBSRC (grants BB/P010598/1, BB/L010569/1, and BB/L010569/2), SFI (grant 16/BBSRC/3390), DAERA (project 13-03-07) and Defra.

## CONFLICT OF INTEREST

R.R.K. is a member of Defra's Science Advisory Council and Bovine TB Partnership, advising government on bovine Tuberculosis control policy.

## AUTHORS' CONTRIBUTIONS

R.R.K. and S.V.G. conceived the study; G.R. and J.C. performed the analyses; T.B. conducted the field epidemiological investigation; G.R., J.C., S.J.L., P.C.L.W. and R.R.K. conceptualised the study; G.R., J.C., T.B., S.J.L., P.C.L.W., S.V.G. and R.R.K. contributed to data interpretation; E.P. and E.L.P. sequenced the bacteria genomes; A.A., R.J.E., R.H., R.S. and G.C.S. provided the data; G.R. and R.R.K. drafted the manuscript. All authors revised the article and approved the final version.

## DATA AVAILABILITY STATEMENT

Restrictions apply to the availability of these data, which belong to Defra and DAERA and were used under license for this study.

## ORCID

*Gianluigi Rossi* https://orcid.org/0000-0002-6540-8467
*Joseph Crispell* https://orcid.org/0000-0002-0364-7112
*Samantha J. Lycett* https://orcid.org/0000-0003-3159-596X
*Piran C. L. White* https://orcid.org/0000-0002-7496-5775
*Adrian Allen* https://orcid.org/0000-0002-9077-8662
*Richard J. Ellis* https://orcid.org/0000-0002-2690-9090
*Stephen V. Gordon* https://orcid.org/0000-0002-4833-5542
*Eleftheria Palkopoulou* https://orcid.org/0000-0001-7382-7509
*Robin Skuce* https://orcid.org/0000-0001-5177-9198
*Graham C. Smith* https://orcid.org/0000-0002-9897-6794
*Rowland R. Kao* https://orcid.org/0000-0003-0919-6401


## REFERENCES


Akhmetova, A., Guerrero, J., McAdam, P., Salvador, L. C. M., Crispell, J., Lavery, J., Presho, E., Kao, R. R., Biek, R., Menzies, F., Trimble, N., Harwood, R., Theo Pepler, P., Oravcova, K., Graham, J., Skuce, R., du Plessis, L., Thompson, S., Wright, L., … Allen, A. R. (2021). Genomic epidemiology of *Mycobacterium bovis* infection in sympatric badger and cattle populations in Northern Ireland. *bioRxiv*. https://doi.org/10.1101/2021.03.12.435101

Anderson, D. P., Ramsey, D. S. L., Nugent, G., Bosson, M., Livingstone, P., Martin, P. A. J., Sergeant, E., Gormley, A. M., & Warburton, B. (2013). A novel approach to assess the probability of disease eradication from a wild-animal reservoir host. *Epidemiology and Infection*, *141*(7), 1509–1521. https://doi.org/10.1017/S0950268812003100X

Anderson, L. G., Gortázar, C., Vicente, J., Hutchings, M. R., & White, P. C. L. (2013). Modelling the effectiveness of vaccination in controlling bovine tuberculosis in wild boar. *Wildlife Research*, *40*(5), 367–376. https://doi.org/10.1071/WR12139

Biek, R., O'Hare, A., Wright, D., Mallon, T., McCormick, C., Orton, R. J., McDowell, S., Trewby, H., Skuce, R. A., & Kao, R. R. (2012). Whole genome sequencing reveals local transmission patterns of *Mycobacterium bovis* in sympatric cattle and badger populations. *PLoS Path*, *8*(11). https://doi.org/10.1371/journal.ppat.1003008

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*(4), 1–6. https://doi.org/10.1371/journal.pcbi.1003537

Brooks-Pollock, E., Roberts, G. O., & Keeling, M. J. (2014). A dynamic model of bovine tuberculosis spread and control in Great Britain. *Nature*, *511*(7508), 228–231. https://doi.org/10.1038/nature13529

Campbell, E. L., Byrne, A. W., Menzies, F. D., McBride, K. R., McCormick, C. M., Scantlebury, M., & Reid, N. (2019). Interspecific visitation of cattle and badgers to fomites: A transmission risk for bovine tuberculosis? *Ecology and Evolution*, *9*(15), 8479–8489. https://doi.org/10.1002/ece3.5282

Campbell, F., Strang, C., Ferguson, N., Cori, A., & Jombart, T. (2018). When are pathogen genome sequences informative of transmission events? *PLoS Path*, *14*(2), 1–21. https://doi.org/10.1371/journal.ppat.1006885

Cassidy, J. P. (2006). The pathogenesis and pathology of bovine tuberculosis with insights from studies of tuberculosis in humans and laboratory animal models. *Veterinary Microbiology*, 151–161. https://doi.org/10.1016/j.vetmic.2005.11.031

Craft, M. E. (2015). Infectious disease transmission and contact networks in wildlife and livestock. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 20140107. https://doi.org/10.1098/rstb.2014.0107

Craft, M. E., Hawthorne, P. L., Packer, C., & Dobson, A. P. (2008). Dynamics of a multihost pathogen in a carnivore community. *Journal of Animal Ecology*, *77*(6), 1257–1264. https://doi.org/10.1111/j.1365-2656.2008.01410.x

Crispell, J., Benton, C. H., Balaz, D., De Maio, N., Ahkmetova, A., Allen, A., Biek, R., Presho, E. L., Dale, J., Hewinson, G., & Lycett, S. J. (2019). Combining genomics and epidemiology to analyse bi-directional transmission of *Mycobacterium bovis* in a multi-host system. *eLife*, 1–36. https://doi.org/10.7554/eLife.45833.001

Crispell, J., Cassidy, S., Kenny, K., McGrath, G., Warde, S., Cameron, H., Rossi, G., MacWhite, T., White, P. C. L., Lycett, S., Kao, R. R., Moriarty, J., & Gordon, S. V. (2020). *Mycobacterium bovis* genomics reveals transmission of infection between cattle and deer in Ireland. *Microbial Genomics*, *6*(8). https://doi.org/10.1099/mgen.0.000388

Crispell, J., Zadoks, R. N., Harris, S. R., Paterson, B., Collins, D. M., de-Lisle, G. W., Livingstone, P., Neill, M. A., Biek, R., Lycett, S. J., Kao, R. R., & Price-Carter, M. (2017). Using whole genome sequencing to investigate transmission in a multi-host system: Bovine tuberculosis in New Zealand. *BMC Genomics*, *18*(1), 180. https://doi.org/10.1186/s12864-017-3569-x

DAERA-NI. (2015). *The test and vaccinate or remove (TVR) wildlife intervention research project – Year 2 report*, https://www.dardni.gov.uk/test-and-vaccinate-or-remove.htm Retrieved from https://www.daera-ni.gov.uk/articles/test-and-vaccinate-or-remove-tvr-wildlife-intervention-research

De Maio, N., Wu, C.-H., O'Reilly, K. M., & Wilson, D. (2015). New routes to phylogeography: A Bayesian structured coalescent approximation. *PLoS Genetics*, *11*(8), 1–22. https://doi.org/10.1371/journal.pgen.1005421

Defra. (2019a). *An update on TB surveillance in wildlife*. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/830810/surveillance-wildlife-2018.pdf

Defra. (2019b). *TB surveillance in badgers during year 1 badger control operations in eastern Cumbria, Low Risk Area (2018)*. Retrieved from https://www.gov.uk/government/publications/bovine-tb-surveillance-in-wildlife-in-england/tb-surveillance-in-badgers-during-year-1-badger-control-operations-in-eastern-cumbria-low-risk-area-2018

Defra. (2020a). *Setting the minimum and maximum numbers in badger cull areas in 2020*. https://doi.org/10.1111/j.1469-7998.2007.00353.x

Defra. (2020b). *TB surveillance in badgers during year 2 badger control operations in eastern Cumbria, Low Risk Area*. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/875548/tb-surveillance-2019-operations.pdf

Defra. (2021). *An update on TB surveillance in wildlife*. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/974351/tb-surveillance-in-wildlife-mar2021.pdf

Defra. (2011). *Bovine TB Eradication Programme for England*. Retrieved from https://www.gov.uk/government/publications/bovine-tb-eradication-programme-for-england

Defra. (2016). *Bovine TB Information Note 03/16 Reducing TB risks from the sale of cattle from 4-yearly testing herds in England*. Retrieved from https://www.gov.uk/government/publications/bovine-tb-information-note-0116-post-movement-testing

Delahay, R. J., Walker, N., Smith, G. S., Wilkinson, D., Clifton-Hadley, R. S., Cheeseman, C. L., Tomlinson, A. J., & Chambers, M. A. (2013). Long-term temporal trends and estimated transmission rates for *Mycobacterium bovis* infection in an undisturbed high-density badger (*Meles meles*) population. *Epidemiology and Infection*, *141*(7), 1445–1456. https://doi.org/10.1017/S0950268813000721

Donnelly, C. A., Woodroffe, R., Cox, D. R., Bourne, F. J., Cheeseman, C. L., Clifton-Hadley, R. S., Wei, G., Gettinby, G., Gilks, P., Jenkins, H., & Johnston, W. T. (2006). Positive and negative effects of widespread badger culling on tuberculosis in cattle. *Nature*, *439*(7078), 843–846. https://doi.org/10.1038/nature04454

Donnelly, C. A., Woodroffe, R., Cox, D. R., Bourne, J., Gettinby, G., Le Fevre, A. M., McInerney, J. P., & Morrison, W. I. (2003). Impact of localized badger culling on tuberculosis incidence in British cattle. *Nature*, *426*(6968), 834–837. https://doi.org/10.1038/nature02192

Drewe, J. A., Tomlinson, A. J., Walker, N. J., & Delahay, R. J. (2010). Diagnostic accuracy and optimal use of three tests for tuberculosis in live badgers. *PLoS ONE*, *5*(6). https://doi.org/10.1371/journal.pone.0011196

Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, *77*(4), 802–813. https://doi.org/10.1111/j.1365-2656.2008.01390.x

Fitzgerald, S. D., & Kaneene, J. B. (2013). Wildlife reservoirs of bovine tuberculosis worldwide: Hosts, pathology, surveillance, and control. *Veterinary Pathology*, *50*(3), 488–499. https://doi.org/10.1177/0300985812467472

Gilbert, M., Mitchell, A., Bourn, D., Mawdsley, J., Clifton-Hadley, R., & Wint, W. (2005). Cattle movements and bovine tuberculosis in Great Britain. *Nature*, *435*(7041), 491–496. https://doi.org/10.1038/nature03548

Godfray, H. C., Donnelly, C. A., Kao, R. R., Macdonald, D. W., McDonald, R. A., Petrokofsky, G., Wood, J. L., Woodroffe, R., Young, D. B., & McLean, A. R. (2013). A restatement of the natural science evidence base relevant to the control of bovine tuberculosis in Great Britain. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1768), 1–18. https://doi.org/10.1098/rspb.2013.1634

Gortazar, C., Diez-Delgado, I., Barasona, J. A., Vicente, J., De La Fuente, J., & Boadella, M. (2015). The wild side of disease control at the wildlife-livestock-human interface: A review. *Frontiers in Veterinary Science*, *1*(January), 1–12. https://doi.org/10.3389/fvets.2014.00027

Green, D. M., Kiss, I. Z., Mitchell, A. P., & Kao, R. R. (2008). Estimates for local and movement-based transmission of bovine tuberculosis in British cattle. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1638), 1001–1005. https://doi.org/10.1098/rspb.2007.1601

Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying reservoirs of infection: A conceptual and practical challenge. *Emerging Infectious Diseases*, *8*(12), 1468–1473. https://doi.org/10.3201/eid0812.010317

Judge, J., Wilson, G. J., Macarthur, R., Delahay, R. J., & McDonald, R. A. (2014). Density and abundance of badger social groups in England and Wales in 2011–2013. *Scientific Reports*, *4*, 1–8. https://doi.org/10.1038/srep03809

Kao, R. R., Haydon, D. T., Lycett, S. J., & Murcia, P. R. (2014). Supersize me: How whole-genome sequencing and big data are transforming epidemiology. *Trends in Microbiology*, *22*(5), 282–291. https://doi.org/10.1016/j.tim.2014.02.011

Keeling, M. J., & Ross, J. V. (2008). On methods for studying stochastic disease dynamics. *Journal of the Royal Society Interface*, *5*(19), 171–181. https://doi.org/10.1098/rsif.2007.1106

Kinsley, A. C., Rossi, G., Silk, M. J., & VanderWaal, K. (2020). Multilayer and multiplex networks: An introduction to their use in veterinary epidemiology. *Frontiers in Veterinary Science*, 596. https://doi.org/10.3389/fvets.2020.00596

Martin, S. W., Eves, J. A., Dolan, L. A., Hammond, R. F., Griffin, J. M., Collins, J. D., & Shoukri, M. M. (1997). The association between the bovine tuberculosis status of herds in the East Offaly Project Area, and the distance to badger setts, 1988–1993. *Preventive Veterinary Medicine*, *31*(1–2), 113–125. https://doi.org/10.1016/S0167-5877(96)01111-7

Nuñez-Garcia, J., Downs, S. H., Parry, J. E., Abernethy, D. A., Broughan, J. M., Cameron, A. R., Cook, A. J., de la Rua-Domenech, R., Goodchild, A. V., Gunn, J., More, S. J., Rhodes, S., Rolfe, S., Sharp, M., Upton, P. A., Vordermeier, H. M., Watson, E., Welsh, M., Whelan, A. O., … Greiner, M. (2018). Meta-analyses of the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis in the UK and Ireland. *Preventive Veterinary Medicine*, *153*, 94–107. https://doi.org/10.1016/j.prevetmed.2017.02.017

Olea-Popelka, F. J., Flynn, O., Costello, E., McGrath, G., Collins, J. D., O'Keeffe, J., Kelton, D. F., Berke, O., & Martin, S. W. (2005). Spatial relationship between *Mycobacterium bovis* strains in cattle and badgers in four areas in Ireland. *Preventive Veterinary Medicine*, *71*(1–2), 57–70. https://doi.org/10.1016/j.prevetmed.2005.05.008

Pollock, J. M., & Neill, S. D. (2002). *Mycobacterium bovis* infection and tuberculosis in cattle. *Veterinary Journal*, *163*(2), 115–127. https://doi.org/10.1053/tvjl.2001.0655

Roper, T. (2010). *Badger*. Collins (Collins New Naturalist Series). Retrieved from https://books.google.co.uk/books?id=FZhrPgAACAAJ

Rossi, G., Crispell, J., Balaz, D., Lycett, S. J., Delahay, R. J., & Kao, R. R. (2020). Identifying likely transmission pairs with pathogen sequence data using Kolmogorov Forward Equations; an application to *M. bovis* in cattle and badgers. *Scientific Reports*, 1–13. https://doi.org/10.1101/2020.06.11.146894

Rossi, G., De Leo, G. A., Pongolini, S., Natalini, S., Vincenzi, S., & Bolzoni, L. (2015). Epidemiological modelling for the assessment of bovine tuberculosis surveillance in the dairy farm network in Emilia-Romagna (Italy). *Epidemics*, *11*, 62–70. https://doi.org/10.1016/j.epidem.2015.02.007

Salvador, L. C., O'Brien, D. J., Cosgrove, M. K., Stuber, T. P., Schooley, A. M., Crispell, J., Church, S. V., Gröhn, Y. T., Robbe-Austerman, S., Kao, R. R. (2019). Disease management at the wildlife-livestock interface: Using whole-genome sequencing to study the role of elk in *Mycobacterium bovis* transmission in Michigan, USA. *Molecular Ecology*, (February), 1–14. https://doi.org/10.1111/mec.15061

Sharkey, K. J. (2008). Deterministic epidemiological models at the individual level. *Journal of Mathematical Biology*, *57*(3), 311–331. https://doi.org/10.1007/s00285-008-0161-7

Silk, M. J., Drewe, J. A., Delahay, R. J., Weber, N., Steward, L. C., Wilson-Aggarwal, J., Boots, M., Hodgson, D. J., Croft, D. P., & McDonald, R. A. (2018). Quantifying direct and indirect contacts for the potential transmission of infection between species using a multilayer contact network. *Behaviour*. https://doi.org/10.1163/1568539X-00003493

Skuce, R., Breadon, E., Allen, A., Milne, G., McCormick, C., Hughes, C., Rutherford, D., Smith, G., Thompson, S., Graham, J., Harwood, R., & Byrne, A. (2020). Longitudinal dynamics of herd-level *Mycobacterium bovis* MLVA type surveillance in cattle in Northern Ireland 2003–2016. *Infection, Genetics and Evolution*, *79*(July 2019), p. 104131. https://doi.org/10.1016/j.meegid.2019.104131

Smith, N. H., Gordon, S. V., de la Rua-Domenech, R., Clifton-Hadley, R. S., & Hewinson, R. G. (2006). Bottlenecks and broomsticks: The molecular evolution of *Mycobacterium bovis*. *Nature Reviews Microbiology*, *4*(9), 670–681. https://doi.org/10.1038/nrmicro1472

Wiethoelter, A. K., Beltrán-Alcrudo, D., Kock, R., & Mor, S. M. (2015). Global trends in infectious diseases at the wildlife–livestock interface. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(31), 9662–9667. https://doi.org/10.1073/pnas.1422741112

Woodroffe, R., Donnelly, C. A., Cox, D. R., Gilks, P., Jenkins, H. E., Johnston, W. T., Le Fevre, A. M., Bourne, F. J., Cheeseman, C. L.,

Clifton-Hadley, R. S., Gettinby, G., Hewinson, R. G., McInerney, J. P., Mitchell, A. P., Morrison, W. I., & Watkins, G. H. (2009). Bovine tuberculosis in cattle and badgers in localized culling areas. *Journal of Wildlife Diseases*, *45*(1), 128–143. https://doi.org/10.7589/0090-3558-45.1.128

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Rossi, G., Crispell, J., Brough, T., Lycett, S. J., White, P. C. L., Allen, A., Ellis, R. J., Gordon, S. V., Harwood, R., Palkopoulou, E., Presho, E. L., Skuce, R., Smith, G. C., & Kao, R. R. (2021). Phylodynamic analysis of an emergent *Mycobacterium bovis* outbreak in an area with no previously known wildlife infections. *Journal of Applied Ecology*, 00, 1–13. https://doi.org/10.1111/1365-2664.14046