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Disease management at the wildlife-livestock interface: using whole-genome sequencing to study the role of elk in Mycobacterium bovis transmission in Michigan, USA

1   **Molecular Ecology**

2   Disease management at the wildlife-livestock interface: using whole-genome sequencing to

3   study the role of elk in *Mycobacterium bovis* transmission in Michigan, USA

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53   Running title (< 45 spaces including spaces)**:** Bovine tuberculosis dynamics in Michigan

54

55   **Abstract (250)**

56   The role of wildlife in the persistence and spread of livestock diseases is difficult to quantify

57   and control. These difficulties are exacerbated when several wildlife species are potentially

58   involved. Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, has experienced an

59   ecological shift in Michigan, with spillover from cattle leading to an endemically infected

60   white-tailed deer (deer) population. It has potentially substantial implications for the health and

61   well-being of both wildlife and livestock and incurs a significant economic cost to industry and

62   government. Deer are known to act as a reservoir of infection, with evidence of *M. bovis*

63   transmission to sympatric elk and cattle populations. However, the role of elk in the circulation

64   of *M. bovis* is uncertain – they are few in number, but range further than deer, so may enable

65   long distance spread. Combining Whole Genome Sequences (WGS) for *M. bovis* isolates from

66   exceptionally well-observed populations of elk, deer and cattle with spatio-temporal locations,

67   we use spatial and Bayesian phylogenetic analyses to show strong spatio-temporal admixture

68   of *M. bovis* isolates. Clustering of bTB in elk and cattle suggests either intraspecies transmission

69   within the two populations, or exposure to a common source. However, there is no support for

70   significant pathogen transfer amongst elk and cattle, and our data are in accordance with

71   existing evidence that interspecies transmission in Michigan is likely only maintained by deer.

72   This study demonstrates the value of whole-genome population studies of *M. bovis*

73   transmission at the wildlife-livestock interface, providing insights into bTB management in an

74   endemic system.

75

Introduction

Use of genomic approaches to understand disease dynamics

In recent years, whole genome sequencing (WGS) technology has created an unprecedented opportunity to study microbial populations and expand the power of traditional epidemiology. It provides insights into pathogen evolution and population structure, sources of pathogen infection, reconstruction of transmission chains, and rates of geographical spread at multiple scales (Drummond et al. 2002; Grenfell et al. 2004; Volz et al. 2009; Pybus and Rambaut 2009; Volz, Koelle, and Bedford 2013; Gire et al. 2014; Kao et al. 2014; Gardy and Loman 2018). While many studies have applied genomic approaches to understand virus evolution, the reduction in cost of WGS technologies have made feasible dense sampling of even much larger bacterial genomes. It has shown that bacterial lineages accumulate sufficient genetic variation over epidemiologically relevant timescales to generate novel insights intro transmission patterns (Biek et al. 2015). Sequence analysis tools such as Bayesian Evolutionary Analysis by Sampling Trees (BEAST) utilize the genetic variation present in sets of samples to estimate evolutionary parameters in the context of time and space (Drummond et al. 2005, 2006; Drummond and Rambaut 2007; Lemey et al. 2009). Reconstruction of pathogen genealogies from time-structured sequence data allows for the estimation of evolutionary substitution rates (molecular clock), which can be used to measure the timing of epidemiologically important events, such as epidemic outbreaks and interspecies transmission (Firth et al. 2010); they also allow us to study infectious diseases in multi-host systems and the identification of pathogen reservoirs (Heled and Drummond 2012; De Maio et al. 2015). It has been shown that ancestral state reconstruction of pathogen genealogies through phylogenetic trees is a useful tool to address this challenge (Heled and Drummond 2012). This approach allows us to estimate the probability of tree internal node states and tree branches being associated with a specific host (and as such the most likely source of infection within the sampled population), based on

101 relationships among the host states at the branch tips (from the sampled isolates), and has

102 provided, for example, evidence that free-ranging elk are currently a self-sustaining brucellosis

103 reservoir and the source of livestock infections in the Great Yellowstone Ecosystem (Kamath

104 et al. 2016).

105

106 **Control of infectious diseases at the wildlife-livestock interface**

107 Infectious diseases at the wildlife-livestock interface endanger the health and well-being of

108 wildlife and livestock. They contribute to considerable economic losses to each sector,

109 including wildlife-related sectors such as hunting and wildlife tourism, and they also represent

110 a potential burden to the whole ecosystem (Wiethoelter et al. 2015; Hassell et al. 2017). The

111 livestock sector is affected through increased mortality and reduced livestock productivity, as

112 well as indirect losses associated with cost of surveillance, decreased market values, food

113 insecurity, and impacts on farmers' livelihood (Dehove et al. 2012).The recreational

114 manipulation of the natural environment to increase the density of wildlife beyond its normal

115 carrying capacity, together with agricultural intensification and deforestation, have resulted in

116 interactions between wildlife and livestock becoming more frequent (Jones et al. 2013; Are R.

117 Berentsen et al. 2014; Lavelle et al. 2016; Skuce et al. 2012; Cowie et al. 2016), creating a

118 dynamic and bidirectional opportunity for pathogens to circulate freely within and across

119 species (Bengis, Kock, and Fischer 2002), via direct and/or indirect routes (use of communal

120 environment, shared resources, etc). The control of infectious diseases at the wildlife-livestock

121 interface is particularly challenging because of the differences in disease control efforts aimed

122 respectively at both livestock and wildlife populations (Gortazar et al. 2015; Bird and Mazet

123 2018), as these are usually managed by different organisational entities (Miller, Farnsworth,

124 and Malmberg 2013; Welburn 2011; Mcbeth and Shanahan 2004).

125

Bovine tuberculosis in a multi-host system in Michigan

Michigan, USA, is one of many places worldwide where the zoonotic disease bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, has become established in free-ranging wildlife (S D Fitzgerald and Kaneene 2013; Palmer 2013; Gortázar, Che Amat, and O'Brien 2015), complicating eradication and control of the disease in cattle. In areas where more than one sympatric wildlife species may be capable of acting as a competent reservoir, determining the roles of the different species in disease maintenance can be both difficult and important, reflecting problems found in many other systems (Haydon et al. 2002; Hlokwe, van Helden, and Michel 2014; Nugent, Gortazar, and Knowles 2015; Shury 2015).

In Michigan, while white-tailed deer (*Odocoileus virginianus*; deer) are well-established as the primary wildlife maintenance host of bTB (Schmitt et al. 1997; O'Brien et al. 2006, 2011; Palmer 2013). In areas where they are sympatric with infected elk (*Cervus elaphus nelsoni*), some uncertainty remains concerning what role, if any, elk play in the epidemiology of the disease (O'Brien et al. 2008). While elk have thus far been assumed to be spillover hosts due to the small number of *M. bovis*-positive animals found to date, they have proven to be capable maintenance hosts in other settings (Fanning and Edwards 1991; Rhyan et al. 1992; Shury and Bergeson 2011). If elk were maintenance hosts in Michigan, management objectives for the population would likely need to shift from conservation for sustained use (hunting and recreational viewing) to disease control. Furthermore, if elk populations are not acting as maintenance hosts they could still play an important role in disease persistence and spread, due to their wide-ranging behaviour relative to deer (Walsh 2007). Evidence for either could entail the need for measures such as density reductions, or issuance of out-of-season shooting permits for animals in close proximity to livestock operations, and exacerbate any social and political conflicts that may exist between wildlife and agricultural interests (O'Brien, Cook, Schmitt,

151 and Jessup 2014).   Moreover, the resources necessary to provide bTB surveillance could

152 escalate disproportionately (Livingstone et al. 2015). Ongoing surveillance of bTB in deer and

153 elk populations has provided valuable information on the prevalence and spatial occurrence of

154 bTB in areas of Michigan where the two species are sympatric. This provides an ideal

155 background for using WGS to identify genetic clustering of isolates. This would be indicative

156 of intraspecies transmission, potentially revealing evidence of maintenance of *M. bovis* in elk,

157 and allowing for estimation of interspecies transmission rates amongst the sampled elk, deer

158 and cattle (*Bos taurus*) populations (Kao et al. 2014).

159

160 **Objectives**

161 In this study, we evaluate the spatial and temporal dynamics of bTB amongst wildlife and

162 livestock in the Lower Peninsula of Michigan. We use WGS to create high resolved time

163 calibrated phylogenies and generate a robust genomic dataset with temporal, spatial and host

164 phenotypic data. Our objectives for this study are to: i) investigate the evolutionary dynamics

165 of *M. bovis* in the Michigan Lower Peninsula; ii) identify *M. bovis* lineages associated with host

166 species and/or geographic locations; iii) quantify the probability *M. bovis* transmission between

167 host species; and iv) gain insights into the needs of a new management program of bTB control

168 at the wildlife-livestock interface. We present data showing three genetically distinct *M. bovis*

169 clades with variable temporal, host and geographical distributions. While elk is present in two

170 out of three clades, no evidence was found for significant transmission between cattle and elk.

171 Our analyses are also consistent with interspecies transmission in Michigan being maintained

172 by deer, and thus the major management focus should continue to be in controlling the disease

173 in the endemic deer population. This study shows the value of WGS for examining bacterial

174 pathogen transmission at the wildlife-livestock interface.

175

Materials and Methods

1. **Data.** *Mycobacterium bovis* isolates were obtained from naturally infected wildlife (deer
and elk) and livestock (cattle) tissue samples using standard isolation protocols (Parish and
Stocker 2002). Wildlife management information, surveillance methods used to find infected
free-ranging wildlife (through hunting and out-of-season shooting permits) and hunting
territories (from where the data were collected) are described in Text S2 and elsewhere
(O'Brien et al. 2002, 2004, 2008; MDNR1 2018; MDNR2 2018), as are the origin of cattle
isolates (Tsao et al. 2014). Because we are focusing on the potential role of elk in the
transmission of bTB amongst the three species, bTB-positive deer that were spatially (within
10 miles of the sampling location of an elk) and temporally close (within three years before or
after the sampled elk date) to each positive elk were selected for inclusion from among the
available archived isolates. The choice of these thresholds was based on the size of the elk's
home range and on the deer's average lifespan in the wild. Different research projects in
Michigan have looked at elk home range use (Ruhl 1984; Beyer 1987; Walsh 2007) and have
found that home ranges of individual elk are highly variable, ranging from 2 to 100 square
miles. It has been shown that there are no habitat barriers to the movement of elk that would
create subpopulations, and therefore there is evidence for only a single elk group (Walsh
2007). To enhance the likelihood of selecting isolates from deer that have been in contact with
elk, we chose the upper end of the elk ranges and selected all deer isolates that were within a
10-mile radius of each elk (encompassing a total area of ~ 314.6 square miles). The average
lifespan of captive deer is 14 years, but in the wild it is typically only two (Tullar 1983),
therefore, we chose a 3-year window around each elk isolate date to improve the opportunities
to capture evidence of direct contact. As we expect animals living in close spatial and
temporal proximity to be more likely to share the same *M. bovis* strains and, should elk and
deer transmit bTB freely between them, this approach would optimize the opportunities to

201 generate well-mixed phylogenies. Some individual elk range further than the core elk range

202 (elk core range and hunting management units are shown in Figures 1 and S1, respectively);

203 therefore, for the cases where isolates were available, positive deer from outlying areas

204 marking the geographic limits of the core habitat occupied by elk were also included, making

205 a total of 39 individuals. To contextualise these data, 78 randomly chosen samples (from 1994

206 to 2013 that fell outside of the previous criteria) were sequenced from the archived list of

207 infected cases. All cattle herds with bTB cases in the same area (three herds, nine individuals)

208 were selected as were cases from two herds that were identified as breakdown sources

209 through trace out investigations. In total we identified isolates from 5 elk, 117 deer and 12

210 individual cattle (Figure 1). Samples from all individual species were collected in the period

211 between 1996 and 2013. The distribution of isolates by year and species is presented in Table

212 S1. Cattle and elk were found positive either in the same year or cattle herds were found

213 infected 1-3 years after elk infected cases. Population size and bTB prevalence information

214 for each host species is presented in Table S2.

215

216 2. **Whole-genome sequencing and SNP analysis**. DNA was collected from *M. bovis* cultures,

217 libraries were prepared using NexteraXT and then sequenced on an Illumina MiSeq using 2 X

218 250 paired end chemistry. Multiple isolates were indexed per lane, providing approximately

219 50-100x coverage per isolate. Raw sequences were aligned to the reference genome AF2122/97

220 (Genbank accession code PRJNA89) using a Burrows-Wheeler Aligner (BWA) (Li and Durbin

221 2009) and Genome Analysis Toolkit 2.5.2 (GATK) (McKenna et al. 2010; DePristo et al. 2011;

222 Van der Auwera et al. 2013). Base quality score recalibration, duplicate removal, single-

223 nucleotide polymorphism (SNP) and indel (insertion or deletion of nucleotides in the genome)

224 discovery and genotyping were applied across all isolates using standard filtering parameters

225 or variant quality score recalibration according to GATK Best Practices recommendations

226   (McKenna et al. 2010; DePristo et al. 2011; Van der Auwera et al. 2013). Sites that fell within

227   Proline-Glutamate (PE) and Proline-Proline-Glutamate (PPE)- polymorphic CG-repetitive

228   sequences (PGRS) were filtered, as well as SNP positions with a phred-scaled quality (QUAL)

229   score for the alternate non-reference allele lower than 150 and allele count (AC) equal to 1 (see

230   https://github.com/USDA-VS/snp_analysis for bioinformatics scripts and Table S3 for

231   sequencing statistics). Integrated Genomics Viewer (IGV) was used to visually validate SNPs,

232   and SNPs with mapping issues or alignment problems were manually filtered.

233

234   3. **Phylogenetic reconstruction of *Mycobacterium bovis*.** Evolutionary relationships among

235   *M. bovis* isolates were generated using a Bayesian coalescent Markov chain Monte Carlo

236   (MCMC) analysis in BEAST 2 (Bouckaert et al. 2014). To verify the existence of temporal

237   signal in the data, we explored the temporal structure of the sequences using the software

238   Tempest (Rambaut et al. 2016) and performed a tip-date randomization test (Firth et al. 2010),

239   where we looked for the absence of overlap between the 95% credible interval of the original

240   rate estimate and any of the date-randomized datasets (Ramsden, Holmes, and Charleston 2008;

241   Duffy and Holmes 2009; Firth et al. 2010; Duchêne et al. 2015) (see Text S1 for analysis

242   description). We used a marginal likelihood estimation (MLE) model selection approach (path

243   sampling (Lartillot and Philippe 2006)) to determine the best-fit nucleotide substitution, clock

244   and demographic models. Two nucleotide substitution models (Hasegawa, Kishino and Yano

245   (HKY, (Hasegawa, Kishino, and Yano 1985)), and General Time Reversible (GTR, (Tavare

246   1986)) that were both supported by the Bayesian information criteria model selection

247   jModeltest 2 (Darriba et al. 2012)) were chosen for model selection. Four molecular clock

248   models (strict, relaxed normal, relaxed exponential, and random local) were evaluated in

249   combination with three coalescent demographic models (constant population size (Drummond

250   et al. 2002; Kingman 1982), Bayesian skyline (Drummond et al. 2005), and Bayesian extended

skyline (Heled and Drummond 2008)). Model performance was evaluated by MLE based on

the average of two runs of path sampling and paired comparisons (of all models to the first

combination: HKY, constant population size and strict clock) of marginal likelihoods using

Bayes Factor (Kass and Raftery 1995). The best-fit model combination was: HKY nucleotide

substitution model with a gamma-distributed rate variation (which enables the evolutionary rate

to vary amongst sites), the uncorrelated exponential relaxed clock model (which allows each

branch of the phylogenetic tree to have its own evolutionary rate), and an extended Bayesian

skyline model (which estimates the demographic function directly from sequence data without

the requirement of pre-choosing the model dimensionality). Two independent MCMC analyses

were run for 100 million generations and posterior distributions were sampled every 10,000

generations. Model parameters were assessed for convergence and satisfactory effective sample

sizes (>200) in Tracer V1.6 (Rambaut et al. 2014). These runs were combined in LogCombiner

v2.4.8 (Drummond and Rambaut 2007) where trees were subsampled as well, and a maximum

credibility tree was estimated (after discarding the first 10% of trees as a burn-in) using

TreeAnnotator v2.2.0 (Drummond and Rambaut 2007). We estimated the overall *M. bovis*

evolutionary rate and the Most Recent Common Ancestor (MRCA) dates for all individual

clades. In this study, we defined a phylogenetic clade as a cluster of individual isolates that was

evolutionary distinct from other clusters and also highly supported (≥0.95).

4. **Spatial and genetic distances between isolates**. To illustrate the spatial distribution of each

phylogenetic clade, the spatial positions of each isolate were plotted and a convex hull (i.e. the

smallest polygon incorporating a given set of points) was drawn around each estimated clade.

To check how clades are distinctively clustered in space, the (Euclidean) spatial distances

between isolates in the estimated and randomly generated clades were computed. For every pair

of clades being compared, 1,000 random points were chosen from each, and spatial distances

276 were computed per random pairs of isolates. This analysis was repeated for the random

277 (permuted) clade assignments and plotted for all clade pairwise comparisons. A k-means

278 analysis was also performed to identify four spatial clusters of isolates. If the clades are

279 distinctively clustered in space, then there will be a large overlap between the spatial positions

280 of these clusters and of the estimated clades. The minimum spatial and genetic (number of

281 different sites between sequences) distances were computed between each pair of isolates and

282 separated by host species interaction. The spatial analyses were implemented in R (RCoreTeam

283 2014) and used the packages maps (Becker and Wilks 2016), maptools (Bivand and Lewin-

284 Koh 2017), and rgeos (Bivand and Rundel 2017), while the genetic analysis used the R package

285 ape (Paradis, Claude, and Strimmer 2004).

286

287 5. **Ancestral state host reconstruction using discrete traits**. Host species were modelled as a

288 discrete trait over the *M. bovis* genealogy by ancestral state inference using Discrete Ancestral

289 Trait Mapping (DATM) in BEAST 2 (Bouckaert et al. 2014). This approach allowed us to

290 estimate the probability of internal node states and branches being associated with a specific

291 host (and as such the most likely source of infection within the sampled *M. bovis* population in

292 elk, deer or cattle), based on relationships among the host states at the branch tips (from the

293 sampled isolates). Host state posterior probabilities (PP) were reported for ancestral nodes up

294 to the most recent common ancestor. All nodes were annotated with their PP values. The three-

295 state analysis (elk=5, deer=117, and cattle=12) estimated over time the posterior probability

296 that a pathogen transition rate between a particular pair of discrete host states was positive. If

297 this probability is high, then the data strongly support a model (evaluated by Bayes' factors) in

298 which a direct pathogen transition between that particular pair of host species can occur.

299 Similarly, the relative transition rate between that pair of host species was also computed. Two

300 MCMC analyses were run for 100 million generations, sampling every 10,000 generations. The

301   BEAST output was analysed using the Tracer v1.6 program (Rambaut et al. 2014). The

302   phylogenetic trees produced by BEAST were subsampled in LogCombiner and annotated using

303   TreeAnnotator v2.2.0 (Drummond and Rambaut 2007), and the maximum clade credibility tree

304   was visualized using the FigTree v1.3.1 program (Drummond and Rambaut 2007). The

305   estimated posterior probabilities of support of transitions between pairs of host species were

306   plotted for all cases. For the cases where the probability was high, providing strong evidence

307   of direct transition between a particular pair of host species, the mean posterior probability of

308   rate changes was presented.

309

310   6. **Phylogenetic tip-host species, down-sampling and extra-elk permutation tests.** To

311   validate the results associated with host state interactions where our models support pathogen

312   transitions between particular pairs of host states, we performed three phylogenetic tests: a) a

313   phylogenetic tip-host species permutation to investigate the extent of pathogen genetic signal

314   in the host populations, b) a down-sampling test to study the impact of different numbers of

315   isolates in host species interactions, and c) a phylogenetic tip-elk permutation test to check the

316   impact of extra elk in host species interactions. In a) this test involved generating 10 new

317   randomized data sets by permutation of sampled host species, performing DATM analysis for

318   each new file with the same settings as section 5, and comparing parameter estimates

319   (probability of pathogen transition between host species) obtained with the initial data set versus

320   the randomized ones. In b) to test the influence of the uneven number of isolates per host species

321   on the results of our analysis, we generated four types of data sets with a different number of

322   sampled host species each (chosen randomly): Subsample A corresponds to 10 data sets of 5

323   elk (all elk isolates), with 5 random samples from each of the available cattle and deer isolates;

324   subsample B corresponds to 10 data sets of 5 elk, 12 cattle (all cattle isolates) and 12 deer

325   randomly sampled from the 117 deer isolates available; subsample C corresponds to 10 data

13

326 sets of 5 elk, 12 cattle, and 36 deer randomly sampled from the 117 deer isolates available; and

327 subsample D corresponds to 10 data sets of 5 elk, 12 cattle, and 76 deer randomly sampled from

328 the 117 deer isolates available. New DATM analyses with the same setting as section 5 were

329 performed for each one of the 10 files of each data set type. Parameter estimates from the 10

330 analyses in each dataset were combined and compared with the original data. These results were

331 shown in boxplots. In c) to identify the effect of under-representation of infected elk in the

332 dynamics of the disease, we have extended our analyses with simulations of 1 and 2 extra elk

333 in the population. We focused on the clades where we have elk and cattle isolates (clades 1-3)

334 and added n elk to our dataset (by randomly replacing the host species labels of n deer by n

335 elk). We repeated this analysis 10 times for n=1 and n=2 (testing the effect of having 6 and 7

336 elk) and computed the probability of support for pathogen transition between each hot species.

337 We compared the results to the original one (with 5 elk).

338

339 **Results**

340 1. **Phylogenetic reconstruction of *Mycobacterium bovis*.** Whole-genome sequencing of the

341 134 *M. bovis* isolates sampled between 1996 and 2013 from multiple hosts (deer, elk and cattle)

342 identified 391 SNPs. An analysis using Tempest supported by tip-date randomization test

343 support the existence of a strong temporal signal in the data (see Text S1 and Figure S2). The

344 time-measured phylogeny, estimated under an uncorrelated relaxed exponential clock and an

345 extended skyline demographic model using BEAST 2 (Figure S3, Table S4), shows three major

346 clades (Figure 2). None of the clades could be distinguished from the others solely by the

347 sampling time of its isolates, nor the area from where they were sampled (Figures 2-3). The

348 spatial distribution of the different clades overlapped with each other to the point where there

349 was no difference between spatial distances calculated between isolates from different clades

350 when these were correctly or randomly assigned (Figure 3A-B). Furthermore, there was no

351 visible relationship (Figure 3-C) between the spatial pattern generated by the three clusters

352 (identified by within group sum of squares in k-means, Figure 3-D) and the one generated by

353 the three clades. These results suggest that different lineages have been co-circulating in the

354 sampled area. The mean evolutionary rate of *M. bovis* was estimated to be 0.37 substitutions

355 per genome per year (95% HPD: 0.24-0.51 substitutions per genome per year) (Figure S4),

356 which is consistent with previous *M. bovis* studies in other settings and with other wildlife hosts

357 (Biek et al. 2012; Trewby et al. 2016; Crispell et al. 2017).

358

359 2. **Investigation of interspecies transmission**. The ancestral host state reconstruction showed

360 that multiple host species were distributed within the different clades, indicative of interspecies

361 transmission (except in clade 3 where deer are the only species present, Figure 4). The

362 clustering patterns of host species observed in clade 2 indicate a strong probability of

363 intraspecies transmission of bTB in the sampled cattle population, while the individual clusters

364 of two elk and two cattle isolates suggest either there are intraspecies transmission events of

365 bTB in the sampled elk and cattle populations, or the infection in each species is due to other

366 common sources. The wide distribution of deer over all the clades suggests that intraspecies

367 transmission is occurring in the sampled deer population and that deer also play an important

368 role in the transmission to other species. State transitions between deer and cattle, and deer and

369 elk were shown to have strong support (PP=0.996 and 0.989, Table 1), but the transition

370 between cattle and elk was poorly supported (PP=0.391, Table 1). When compared to all

371 isolates, cattle and elk isolates were never the closest genetically or spatially to each other

372 (Figures S5-6).

373

374 3. **Phylogenetic tip-host species, down-sampling and extra-elk permutation tests.** To check

375 the veracity of our results we performed host-tip randomization, down-sampling and additional

376   elk analyses. Figure 5 shows that the estimated interactions between deer and elk, and cattle

377   and elk with the real data (presented by stars) differ from the ones estimated with the

378   randomized data sets (presented by boxplots), with the exception of the estimated interactions

379   between cattle and deer from the real and random data sets, which overlap with each other. This

380   overlap suggests that pathogen migration between these two species is consistent with it being

381   a random process. If this is the case, then increases of bTB in cattle may simply be attributable

382   to increases in deer population densities and infection levels. The sensitivity analyses to show

383   the effect of sample size (of each host species) on the interspecies interaction, show that this

384   measure only influences our results under extreme down-sampling (dataset A). However, with

385   lesser but still substantial down-sampling of data (B, C and D; which have variations in sample

386   size for each host species), our analyses show a similar pattern to the original data: strong

387   support for interactions between deer and cattle and deer and elk, and low support for

388   interactions between cattle and elk (Figure 6, Table 1). Finally, the addition of 1 or 2 "elk"

389   samples to our pool of isolates (by replacing deer isolates) were shown to be insufficient to

390   change our results (Figure S7).

391

392   **Discussion**

393   This analysis is one of the few genomic studies examining bacterial transmission at the wildlife-

394   livestock interface (Kamath et al. 2016) in the United States and highlights the important role

395   that genomics and phylodynamic approaches play in improving our understanding of fine scale

396   transmission patterns. Using *M. bovis* genomic data from different host species with a time

397   frame of 17 years, we showed that, even with a slow, highly variable substitution rate, WGS

398   has remarkable power to identify the likely roles of different host species in the transmission

399   dynamics of endemically circulating diseases, independent of other epidemiological evidence.

400   However, with chronic diseases such as bTB (months to years to show signs of infection), we

401   have to consider the possibility of infections that were missed during testing, and that we could

402   be underestimating the amount of transmission. Furthermore, any interpretation of the results

403   should take into consideration the assumptions and limitations of the data and models used in

404   the study. DATMs assume sampling numbers are informative of the underlying prevalence of

405   the disease in different hosts. We have a low sampling number of isolates from elk and cattle

406   and a large number from deer, however, we present information on population sizes and number

407   of sampled and infected cases for each species, demonstrating that our samples are related to

408   the underlying levels of the disease for each host species. Furthermore, the sampling effort in

409   the elk is very high given the proportion of individuals tested relative to the total population

410   size. These models also assume homogeneous mixing in the underlying sampled population,

411   which was addressed by choosing high number of random deer isolates. However, for future

412   studies with structured populations, the adoption of methods like the Bayesian Structured

413   Coalescent Approximation (BASTA) (De Maio et al. 2015), which relaxes that assumption,

414   might be more suitable. Michigan has an unprecedent surveillance system for elk – since their

415   introduction to the state in 1918, they have been heavily managed to ensure a healthy and stable

416   population size (~800 individuals) (MDNR3, n.d.) but even with such a system a few infected

417   cases might have been missed. We showed that even if infected elk were under sampled by

418   40% compared to deer (i.e. two more infected elk), the additional interactions do not alter the

419   key conclusion; however additional analysis would be needed to determine how many more elk

420   would be needed to see an effect. Spatial analyses show that even with the addition of a large

421   random sample of infected deer, disease transmission events occur at small spatial scales with

422   circulation of distinct strains. The spatial overlap of the clades supports the idea that the

423   pathogen population is well mixed (at this scale). Furthermore, *M. bovis'* low and variable

424   substitution rates can sometimes challenge accurate estimations of evolutionary rates. Our

425   estimates of *M. bovis* evolutionary rate for this sampled population is similar with the ones

426 found in other studies with different organisms (Biek et al. 2012; Trewby et al. 2016; Crispell

427 et al. 2017).

428

429 Our results suggest that in the Michigan bTB endemic situation to date, elk so far are unlikely

430 to be a maintenance reservoir. The lack of support of pathogen transition between elk and cattle

431 also suggest that elk do not have an active role in the transmission of *M. bovis* infections to the

432 neighbouring livestock populations. These genomic findings support conclusions based on

433 previously reported pathologic and epidemiologic data (O'Brien et al. 2006, 2008). Overall,

434 the topology of the *M. bovis* phylogeny indicated the existence of interspecies transmission

435 events, with the presence of multiple host species interspersed within clades. Deer isolates were

436 found in all 3 clades, showing that in our selection of isolates there is higher genetic diversity

437 circulating in this host population than in any other, adding to the accumulated evidence from

438 previous ecological studies that deer are a significant source of bTB in livestock and other

439 wildlife species. However, the clustering of isolates by host species suggest the majority

440 of transmission events were occurring either within species, or from a common source,

441 (exposure to the sampled deer population or other intermediate hosts (Lavelle et al. 2016)), or

442 both. For the Michigan elk population, if any of the clustering is due to intraspecies

443 transmission, this is a new and epidemiologically significant finding. If the clustering of

444 infected elk noted in Clade 2 of our study is due to elk-to-elk transmission, it may be that

445 transmission has not yet reached a sufficient threshold for self-maintenance. That said, if

446 intraspecies transmission has occurred at all it should serve as warning to state wildlife

447 managers of the necessity of preventing further introductions of *M. bovis* into that valuable

448 population. Thus, human-caused aggregations (such as recreational feed and bait sites intended

449 for deer) that act as sources of indirect contact between elk and deer must not be allowed to

450 occur.

451

452 In Canada, wild elk have proven to be competent maintenance hosts for bTB (Manitoba

453 Agriculture Department, n.d.; Shury and Bergeson 2011; Shury 2015). Reasons why elk in

454 Canada are maintenance hosts and in Michigan they seem not to be, are not clear, however, are

455 likely to be related to different population sizes, densities, social behaviour and home ranges.

456 In Canada, populations are likely to be larger and denser and composed of multiple groups,

457 while in Michigan they are smaller, and without structured groups and they only overlap slightly

458 with the deer endemic area (Walsh 2007; Shury and Bergeson 2011; Shury 2015). Other factors

459 such as management practices, historical facts of bTB (especially, how long the area has been

460 infected), habitat quality, and opportunities to have inter- and intra- species contact may also

461 play a role in the persistence of *M. bovis* in these populations.

462

463 We also demonstrated via DATM, and genetic and spatial isolate pairwise comparisons, that

464 there is very low support for transition events between elk and cattle. This might be due to the

465 fact that the elk population is an order of magnitude smaller than the deer population, which

466 may decrease the probability of contact with livestock. In addition, much of the core elk range

467 in Michigan is composed of publicly-owned lands that are relatively remote from livestock

468 operations. These findings suggest that bTB eradication efforts in the elk population are

469 currently unnecessary due to the low probability of spillover to cattle, and that the major focus

470 should continue to be in controlling the disease in the endemic deer population. However,

471 should the elk population increase, this could enhance their role in the maintenance of bTB in

472 Michigan. Furthermore, the possibility of other species acting as intermediate hosts and being

473 involved in the transmission of *M. bovis* to the cattle population remains possible. Other

474 spillover hosts including black bear (*Ursus americanus*), bobcat (*Felis rufus*) coyote (*Canis*

475 *latrans*), red fox (*Vulpes vulpes*), raccoons (*Procyon lotor*), and opossums (*Didelphis*

476 *virginiana*) have been shown to be bTB spillovers in this area (Bruning-Fann et al. 2001; Walter

477 et al. 2013; A. R. Berentsen et al. 2011). It could be though direct contact (however unlikely

478 (Scott D. Fitzgerald et al. 2003)), or through environment contamination. Both raccoons and

479 opossums are found to share communal dens resulting in increased interaction when resources

480 are abundant such as around feed stockpiled for livestock (Palmer, Waters, and Whipple 2002;

481 Atwood et al. 2009), and when they have a chance, they use the same stored feed, water sources,

482 and feed being consumed by cattle (Bruning-Fann et al. 2001; Atwood et al. 2009; Walter et al.

483 2013), increasing the chances of contamination. More studies on these populations would help

484 to understand their contribution to the spread of bTB.

485

486 In Michigan, bTB has been a concern of management by both wildlife and agriculture agencies

487 for two decades. Prospects for eradication are uncertain, and the ongoing costs of disease

488 management necessitate the use of innovative methods to inform management decisions. By

489 providing insights into reservoir status and the likelihood of interspecies transmission, genomic

490 analyses such as this supplement traditional epidemiologic and pathologic data, advancing

491 efficient and effective use of both bTB surveillance and control resources.

492

493 **Data Accessibility**

494 The raw sequence files (FASTQ) were submitted to the NCBI Sequence Read Archive under

495 the Bioproject accession number: PRJNA251692. The individual isolates can be accessed

496 under the following Biosample accession numbers: SAMN07339977 - SAMN07340029 and

497 SAMN10254813 – SAMN10254893. Information about metadata associated to each isolate

498 is in Table S3. The R scripts used for this publication are freely available on the following

499 Github link: https://github.com/lsalvador/WGS_Michigan_Project.

500

Author Contributions

LCMS designed the study, performed research, analysed data, and wrote first draft. DJOB designed the study, collected data from wildlife, contributed to interpretation and wrote first draft. MKC designed the study, collected data from wildlife, contributed to interpretation and performed GIS analysis. RRK developed the initial project proposal, designed the study, advised on its implementation and contributed to interpretation. AS and SC revived archived *M. bovis* isolates. SRA sequenced and provided genomic data and livestock metadata. TPS performed bioinformatics analysis. YTG developed the initial project proposal and provided input on the study design. JC provided useful insights on molecular evolution analysis and performed the spatial analysis. MKC, SRA, TPS, JC, YTG and RRK provided comments on the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

527

Host species interaction	Estimated posterior probability of transition between host species (symmetric)	Estimated absolute transition between host species (event/genome/year)	Strength of support by Bayes' factor (BF > 3: well supported BF > 10: very strong support)
Cattle-Deer	**0.996**	**0.012**	**28.37**
Cattle-Elk	0.391	0.011	0.073
Deer-Elk	**0.989**	**0.011**	**10.24**

Table 1. Evidence of pathogen transition between host species. Results from a discrete ancestral trait mapping analysis.
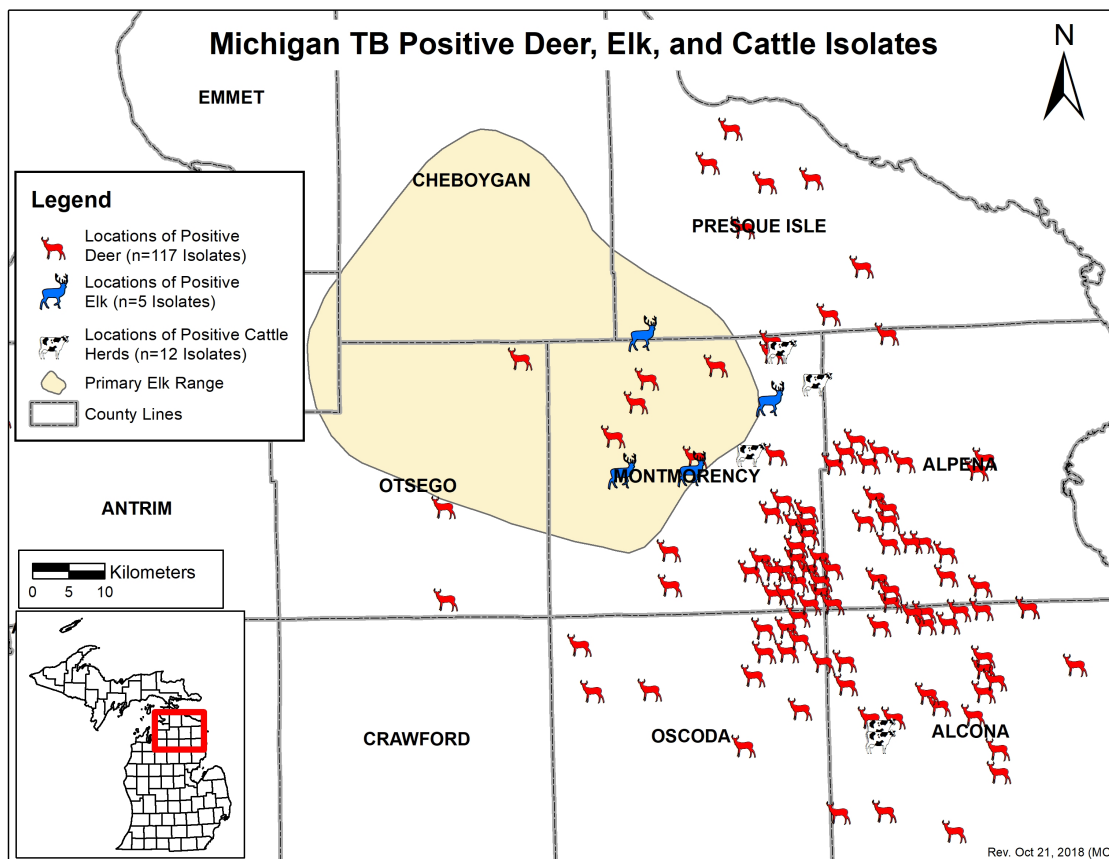
Figure 1. Study area in northeastern Lower Peninsula of Michigan, USA with locations of bovine tuberculosis positive animals. Positive samples from deer that were spatially and temporally close to each positive elk and from the margins of the occupied elk range were selected for inclusion from among available archived isolates (39). This dataset was extended with 78 more positive deer samples randomly chosen from the available archived isolates. Positive cattle herds in the same area (9) were also selected together with trace backs of infected individuals from other herds (3). In total isolates from 5 elk (from 2000 to 2006), 117 deer (from 1996 to 2013) and 12 individual cattle (from 2000 to 2009) from 3 neighbouring herds

and 2 other herds identified by trace backs. The isolates were sampled from 8 counties: Montmorency, Presque Isle, Otsego, Oscoda, Alpena, Alcona, Emmet and Antrim. Isolates that were collected from the same host species in the same location are overlapped in the figure.
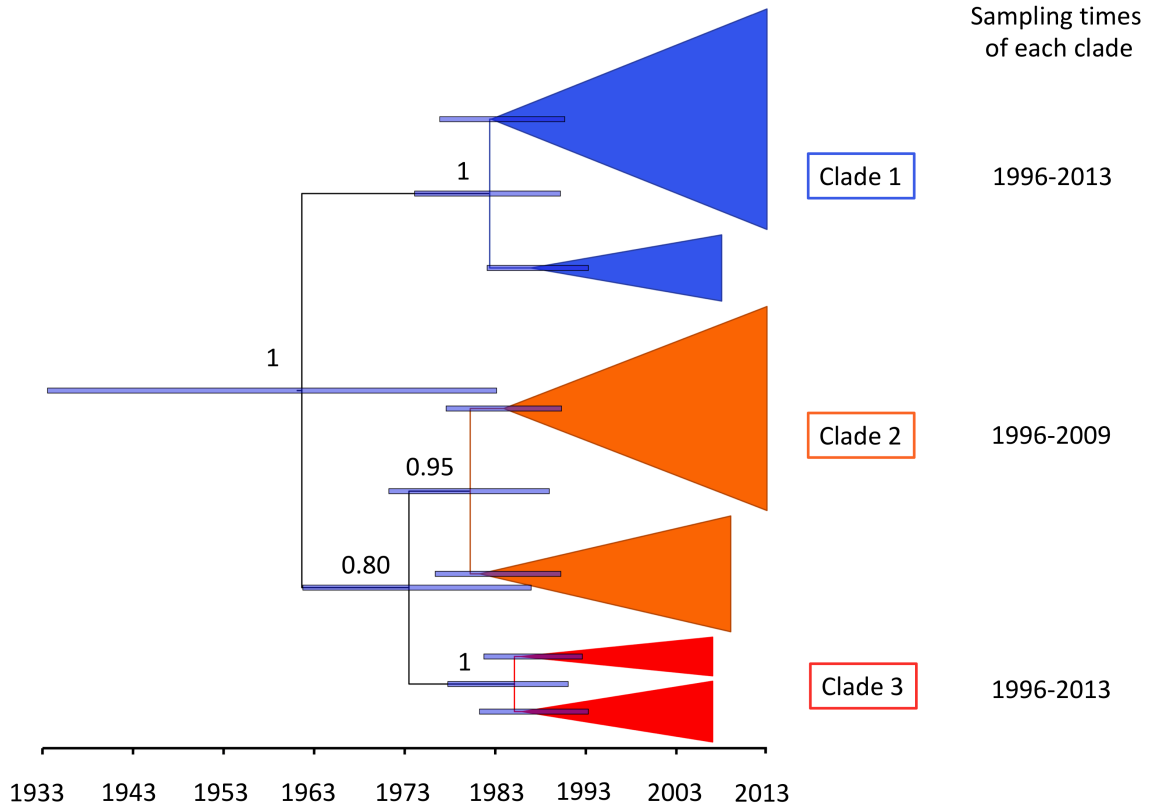
Figure 2. Time-calibrated maximum clade credibility tree of *Mycobacterium bovis* isolates. Four *M. bovis* clades (C1-C3) were identified through Bayesian phylogenetic analyses of 117 *M. bovis* isolates sampled between 1996 and 2013 under an uncorrelated relaxed exponential clock and extended skyline demographic model. Posterior support for major nodes is shown with grey bars indicating the 95% highest posterior density intervals for node date estimates.
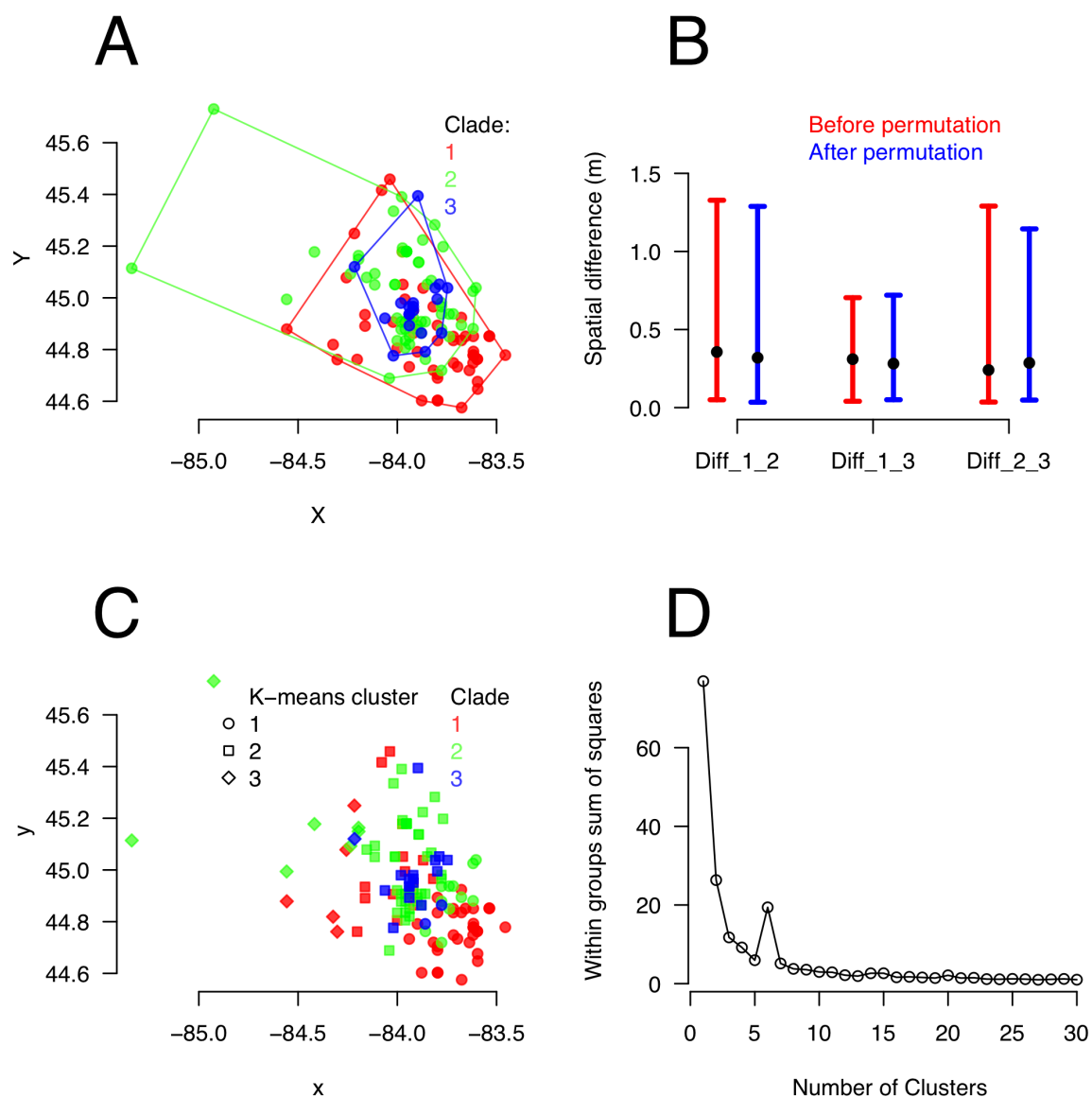
553
554 Figure 3. Spatial analysis of *Mycobacterium bovis* isolates. A. Spatial analysis of distribution
555 of *M. bovis* clades identified by Bayesian phylogenetic analysis. Each polygon represents the
556 minimum convex polygon of the sampled locations of the isolates of each clade. B.
557 Comparison of spatial distances between estimated and permuted clades. For every pair of
558 clades being compared we have randomly selected 1000 isolates from each. For each random pair
559 of isolates we calculated the spatial distance between them. This analysis was repeated with
560 random (permuted) clade assignments. C. K-means analysis with 3 clusters (represented by
561 symbols) versus 3 clades (represented by colors). D. Optimal number of clusters estimated by
562 within group sum of squares (distances between individuals within each cluster). The optimal
563 number of clusters will be the number after which within cluster differences become minimal;
564 here this occurs after ~ 3 clusters.
565
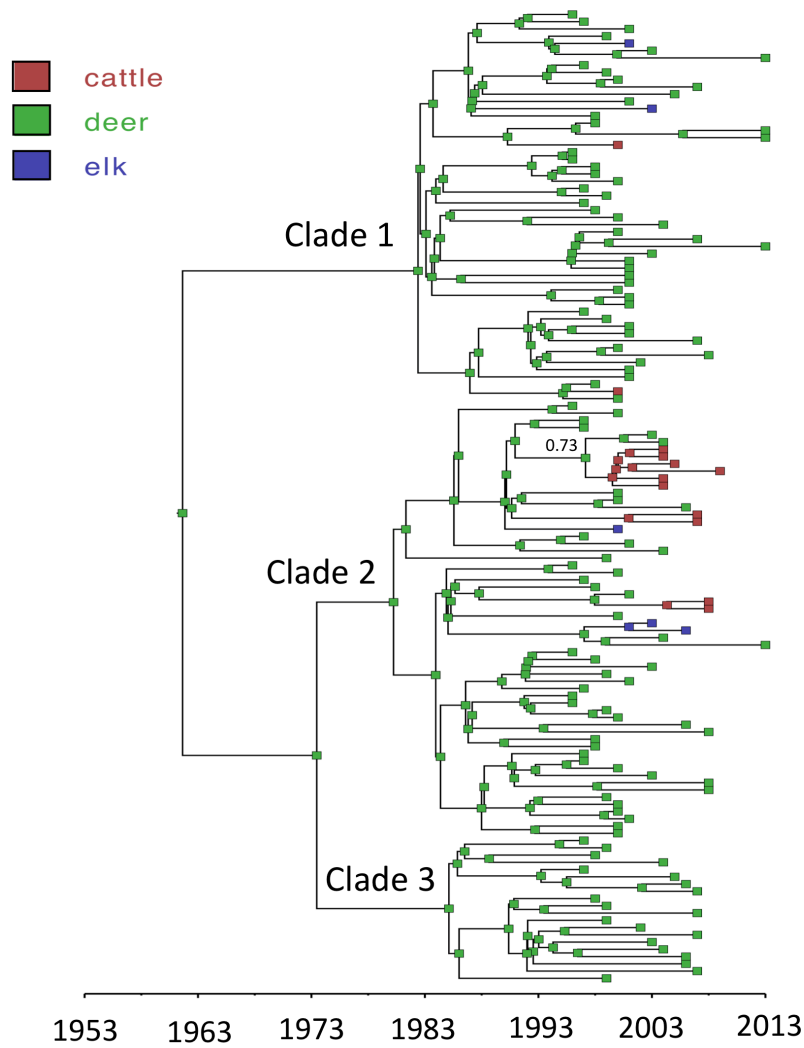
Figure 4. Ancestral host state reconstruction over the *Mycobacterium bovis* phylogeny. Maximum credibility tree was estimated under a model of symmetric host species transitions. Host state posterior probabilities (PP) are reported for ancestral nodes up to the most recent common ancestor. All nodes have PP values above 0.95 and only one (with PP=0.73) is annotated. Host species are represented by squares with the following colour labels (cattle=red, deer=green, elk=blue).
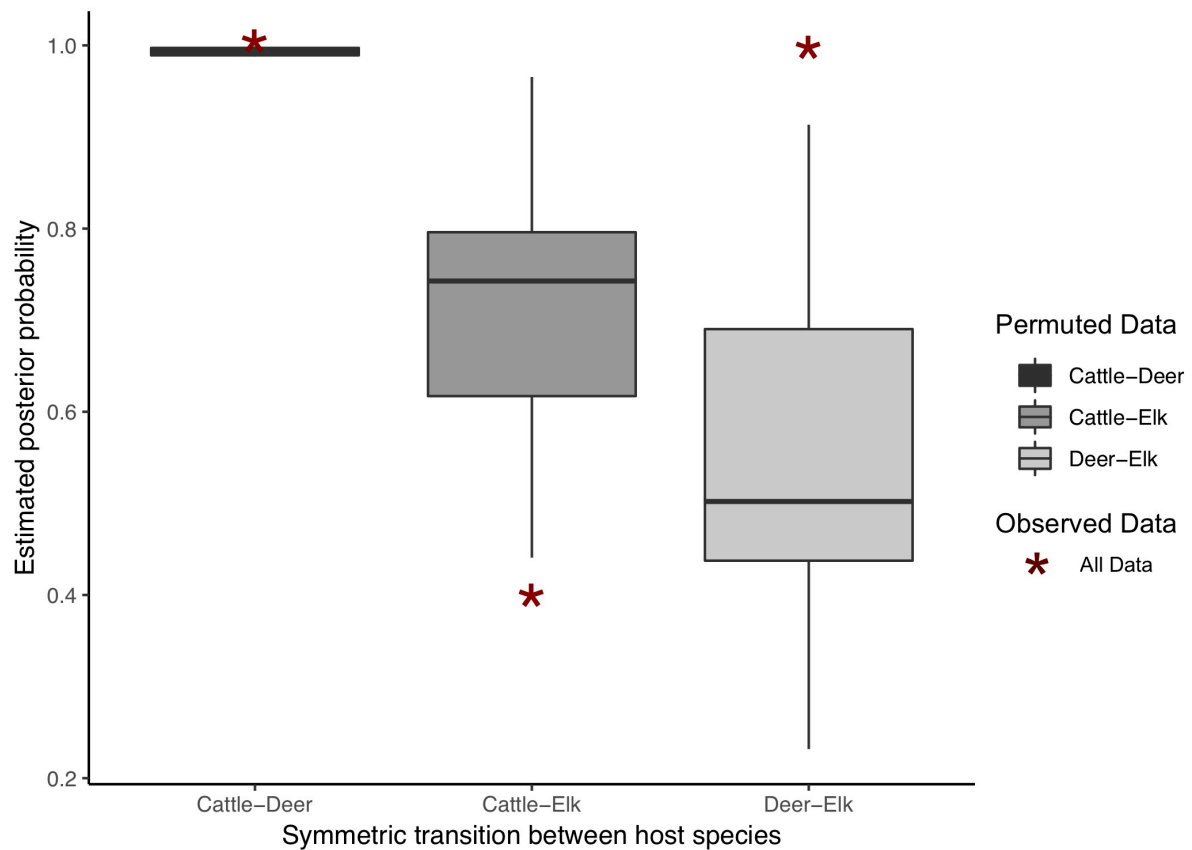
574

575
576 Figure 5. Comparison of the estimated posterior support of direct host species transition
577 between permuted and observed data. The estimated posterior mean probability of each host
578 species interaction is the posterior probability that a particular transition rate is positive. If this
579 probability is high, the data strongly support a model in which there is direct pathogen transition
580 between that particular pair of host species. The posterior means were estimated via a Discrete
581 Ancestral Trait Mapping performed in BEAST v2. The 'Permuted data' correspond to the
582 posterior means of 10 BEAST runs of each interaction after permuting the host species labels
583 each time. The 'Observed data' correspond to the posterior mean of each interaction using the
584 observed data.
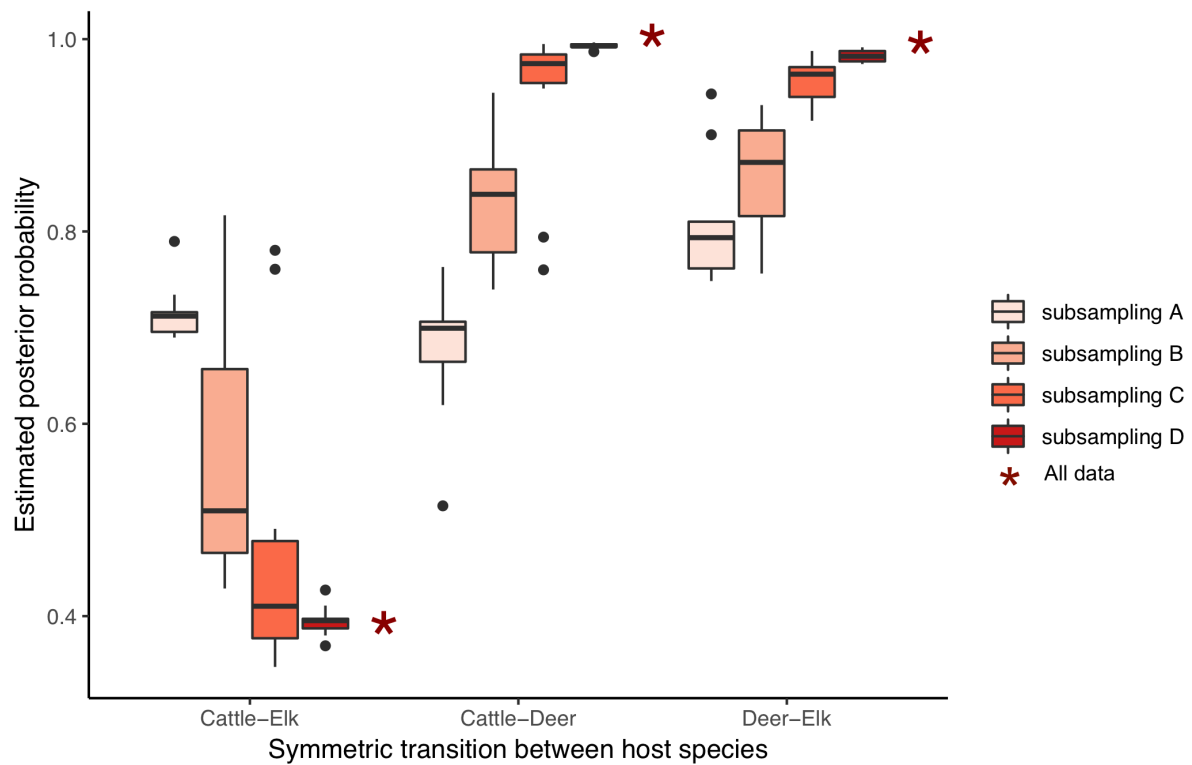
Figure 6. Comparison of the estimated posterior support of direct host species transition between subsampled and observed data. The estimated posterior mean probability of each interaction is the posterior probability that a particular transition rate is positive. If this probability is high, then the data strongly support a model in which there is direct pathogen transition between that particular pair of host species. The posterior means were estimated via a Discrete Ancestral Trait Mapping performed in BEAST v2. The 'Subsampled data' correspond to three subsets of 10 files where the different isolates found in each species were randomly chosen to be part of the new data set. Subsample A corresponds to isolates sampled from five elk ('Elk'), five randomly chosen cattle ('Cattle'), and five randomly chosen deer ('Deer'); Subsample B corresponds to isolates sampled from five elk, nine cattle, and nine randomly chosen deer; and Subsample C corresponds to isolates sampled from five elk, nine cattle, and twenty four randomly chosen deer. The 'All data' correspond to the posterior mean of each host species interaction output by one DATM analysis using all of the observed data, which consists of five elk, twelve cattle, and 117 deer.

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