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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

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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

4  
5 L.C.M. Salvador<sup>1,2,3,4</sup>, D.J. O'Brien<sup>5</sup>, M.K. Cosgrove<sup>5</sup>, T. P. Stuber<sup>6</sup>, A. Schooley<sup>7</sup>, J.  
6 Crispell<sup>8</sup>, S. Church<sup>7</sup>, Y.T., Grohn<sup>9</sup>, S. Robbe-Austerman<sup>6</sup>, R.R. Kao<sup>1,3</sup>

7  
8 <sup>1</sup>Boyd Orr Centre for Population and Ecosystem Health  
9 Institute of Biodiversity, Animal Health and Comparative Medicine  
10 College of Medical, Veterinary and Life Sciences  
11 University of Glasgow, Glasgow, UK

12  
13 <sup>2</sup>Ecology and Evolutionary Biology Department  
14 Princeton University, Princeton, NJ, USA

15  
16 <sup>3</sup>Royal (Dick) Veterinary School of Veterinary Studies  
17 University of Edinburgh, Midlothian, UK

18  
19 <sup>4</sup>Department of Infectious Diseases, College of Veterinary Medicine  
20 Institute of Bioinformatics  
21 University of Georgia, Athens, GA, USA

22  
23 <sup>5</sup>Wildlife Disease Laboratory  
24 Michigan Department of Natural Resources  
25 Lansing, MI, USA

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<sup>6</sup> National Veterinary Services Laboratories  
United States Department of Agriculture  
Animal and Plant Health Inspection Service, Ames, IA, USA

<sup>7</sup> Mycobacteriology Laboratory, Infectious Disease Division  
Michigan Department of Health and Human Services  
Lansing, MI, USA

<sup>8</sup> School of Veterinary Medicine  
College of Health and Agricultural Sciences  
University College Dublin  
Republic of Ireland

<sup>9</sup> Department of Population Medicine and Diagnostic Sciences  
College of Veterinary Medicine  
Cornell University, Ithaca, NY, USA

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Corresponding Author: Rowland R. Kao  
Royal (Dick) Veterinary School of Veterinary Studies  
University of Edinburgh, Midlothian, UK  
Easter Bush Campus, Midlothian, EH25 9RG, UK

51 Email: Rowland.Kao@ed.ac.uk

52

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

76 **Introduction**

77 **Use of genomic approaches to understand disease dynamics**

78 In recent years, whole genome sequencing (WGS) technology has created an unprecedented  
79 opportunity to study microbial populations and expand the power of traditional epidemiology.  
80 It provides insights into pathogen evolution and population structure, sources of pathogen  
81 infection, reconstruction of transmission chains, and rates of geographical spread at multiple  
82 scales ( Drummond et al. 2002; Grenfell et al. 2004; Volz et al. 2009; Pybus and Rambaut 2009;  
83 Volz, Koelle, and Bedford 2013; Gire et al. 2014; Kao et al. 2014; Gardy and Loman 2018).  
84 While many studies have applied genomic approaches to understand virus evolution, the  
85 reduction in cost of WGS technologies have made feasible dense sampling of even much larger  
86 bacterial genomes. It has shown that bacterial lineages accumulate sufficient genetic variation  
87 over epidemiologically relevant timescales to generate novel insights into transmission  
88 patterns (Biek et al. 2015). Sequence analysis tools such as Bayesian Evolutionary Analysis by  
89 Sampling Trees (BEAST) utilize the genetic variation present in sets of samples to estimate  
90 evolutionary parameters in the context of time and space (Drummond et al. 2005, 2006;  
91 Drummond and Rambaut 2007; Lemey et al. 2009). Reconstruction of pathogen genealogies  
92 from time-structured sequence data allows for the estimation of evolutionary substitution rates  
93 (molecular clock), which can be used to measure the timing of epidemiologically important  
94 events, such as epidemic outbreaks and interspecies transmission (Firth et al. 2010); they also  
95 allow us to study infectious diseases in multi-host systems and the identification of pathogen  
96 reservoirs (Heled and Drummond 2012; De Maio et al. 2015). It has been shown that ancestral  
97 state reconstruction of pathogen genealogies through phylogenetic trees is a useful tool to  
98 address this challenge (Heled and Drummond 2012). This approach allows us to estimate the  
99 probability of tree internal node states and tree branches being associated with a specific host  
100 (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

126 **Bovine tuberculosis in a multi-host system in Michigan**

127 Michigan, USA, is one of many places worldwide where the zoonotic disease bovine  
128 tuberculosis (bTB), caused by *Mycobacterium bovis*, has become established in free-ranging  
129 wildlife (S D Fitzgerald and Kaneene 2013; Palmer 2013; Gortázar, Che Amat, and O'Brien  
130 2015), complicating eradication and control of the disease in cattle. In areas where more than  
131 one sympatric wildlife species may be capable of acting as a competent reservoir, determining  
132 the roles of the different species in disease maintenance can be both difficult and important,  
133 reflecting problems found in many other systems (Haydon et al. 2002; Hlokwe, van Helden,  
134 and Michel 2014; Nugent, Gortazar, and Knowles 2015; Shury 2015).

135

136 In Michigan, while white-tailed deer (*Odocoileus virginianus*; deer) are well-established as the  
137 primary wildlife maintenance host of bTB (Schmitt et al. 1997; O'Brien et al. 2006, 2011;  
138 Palmer 2013). In areas where they are sympatric with infected elk (*Cervus elaphus nelsoni*),  
139 some uncertainty remains concerning what role, if any, elk play in the epidemiology of the  
140 disease (O'Brien et al. 2008). While elk have thus far been assumed to be spillover hosts due  
141 to the small number of *M. bovis*-positive animals found to date, they have proven to be capable  
142 maintenance hosts in other settings (Fanning and Edwards 1991; Rhyan et al. 1992; Shury and  
143 Bergeson 2011). If elk were maintenance hosts in Michigan, management objectives for the  
144 population would likely need to shift from conservation for sustained use (hunting and  
145 recreational viewing) to disease control. Furthermore, if elk populations are not acting as  
146 maintenance hosts they could still play an important role in disease persistence and spread, due  
147 to their wide-ranging behaviour relative to deer (Walsh 2007). Evidence for either could entail  
148 the need for measures such as density reductions, or issuance of out-of-season shooting permits  
149 for animals in close proximity to livestock operations, and exacerbate any social and political  
150 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



176 **Materials and Methods**

177 1. **Data.** *Mycobacterium bovis* isolates were obtained from naturally infected wildlife (deer  
178 and elk) and livestock (cattle) tissue samples using standard isolation protocols (Parish and  
179 Stocker 2002). Wildlife management information, surveillance methods used to find infected  
180 free-ranging wildlife (through hunting and out-of-season shooting permits) and hunting  
181 territories (from where the data were collected) are described in Text S2 and elsewhere  
182 (O'Brien et al. 2002, 2004, 2008; MDNR1 2018; MDNR2 2018), as are the origin of cattle  
183 isolates (Tsao et al. 2014). Because we are focusing on the potential role of elk in the  
184 transmission of bTB amongst the three species, bTB-positive deer that were spatially (within  
185 10 miles of the sampling location of an elk) and temporally close (within three years before or  
186 after the sampled elk date) to each positive elk were selected for inclusion from among the  
187 available archived isolates. The choice of these thresholds was based on the size of the elk's  
188 home range and on the deer's average lifespan in the wild. Different research projects in  
189 Michigan have looked at elk home range use (Ruhl 1984; Beyer 1987; Walsh 2007) and have  
190 found that home ranges of individual elk are highly variable, ranging from 2 to 100 square  
191 miles. It has been shown that there are no habitat barriers to the movement of elk that would  
192 create subpopulations, and therefore there is evidence for only a single elk group (Walsh  
193 2007). To enhance the likelihood of selecting isolates from deer that have been in contact with  
194 elk, we chose the upper end of the elk ranges and selected all deer isolates that were within a  
195 10-mile radius of each elk (encompassing a total area of ~ 314.6 square miles). The average  
196 lifespan of captive deer is 14 years, but in the wild it is typically only two (Tullar 1983),  
197 therefore, we chose a 3-year window around each elk isolate date to improve the opportunities  
198 to capture evidence of direct contact. As we expect animals living in close spatial and  
199 temporal proximity to be more likely to share the same *M. bovis* strains and, should elk and  
200 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](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

251 skyline (Heled and Drummond 2008)). Model performance was evaluated by MLE based on  
252 the average of two runs of path sampling and paired comparisons (of all models to the first  
253 combination: HKY, constant population size and strict clock) of marginal likelihoods using  
254 Bayes Factor (Kass and Raftery 1995). The best-fit model combination was: HKY nucleotide  
255 substitution model with a gamma-distributed rate variation (which enables the evolutionary rate  
256 to vary amongst sites), the uncorrelated exponential relaxed clock model (which allows each  
257 branch of the phylogenetic tree to have its own evolutionary rate), and an extended Bayesian  
258 skyline model (which estimates the demographic function directly from sequence data without  
259 the requirement of pre-choosing the model dimensionality). Two independent MCMC analyses  
260 were run for 100 million generations and posterior distributions were sampled every 10,000  
261 generations. Model parameters were assessed for convergence and satisfactory effective sample  
262 sizes ( $>200$ ) in Tracer V1.6 (Rambaut et al. 2014). These runs were combined in LogCombiner  
263 v2.4.8 (Drummond and Rambaut 2007) where trees were subsampled as well, and a maximum  
264 credibility tree was estimated (after discarding the first 10% of trees as a burn-in) using  
265 TreeAnnotator v2.2.0 (Drummond and Rambaut 2007). We estimated the overall *M. bovis*  
266 evolutionary rate and the Most Recent Common Ancestor (MRCA) dates for all individual  
267 clades. In this study, we defined a phylogenetic clade as a cluster of individual isolates that was  
268 evolutionary distinct from other clusters and also highly supported ( $\geq 0.95$ ).

269

270 **4. Spatial and genetic distances between isolates.** To illustrate the spatial distribution of each  
271 phylogenetic clade, the spatial positions of each isolate were plotted and a convex hull (i.e. the  
272 smallest polygon incorporating a given set of points) was drawn around each estimated clade.  
273 To check how clades are distinctively clustered in space, the (Euclidean) spatial distances  
274 between isolates in the estimated and randomly generated clades were computed. For every pair  
275 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

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 host 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 unprecedented 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/l salvador/WGS\\_Michigan\\_Project](https://github.com/l salvador/WGS_Michigan_Project).

500

501 **Author Contributions**

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

512

513 **Conflict of Interest**

514 The authors declare no conflict of interest.

515

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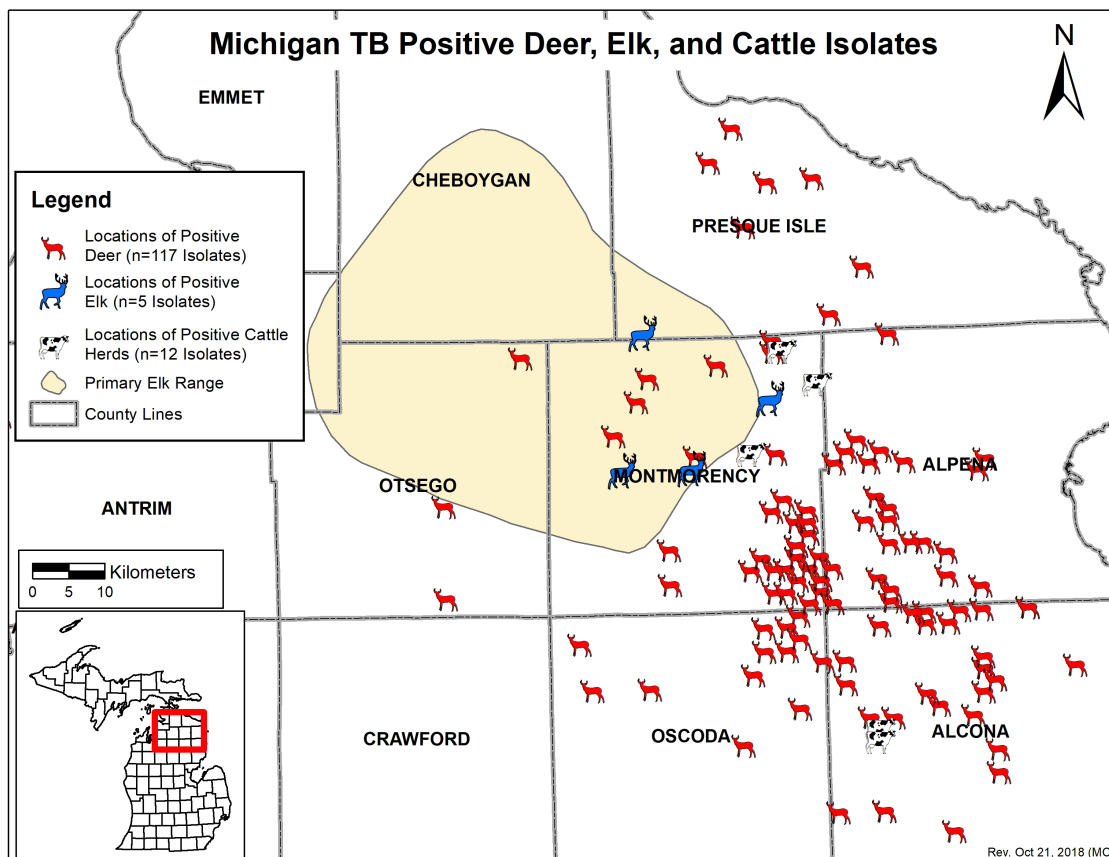
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526 Award No. BB/M01262X/1 and NSF Project No. NYCV478531.  
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Tables and Figures

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)
<b>Cattle-Deer</b>	<b>0.996</b>	<b>0.012</b>	<b>28.37</b>
Cattle-Elk	0.391	0.011	0.073
<b>Deer-Elk</b>	<b>0.989</b>	<b>0.011</b>	<b>10.24</b>

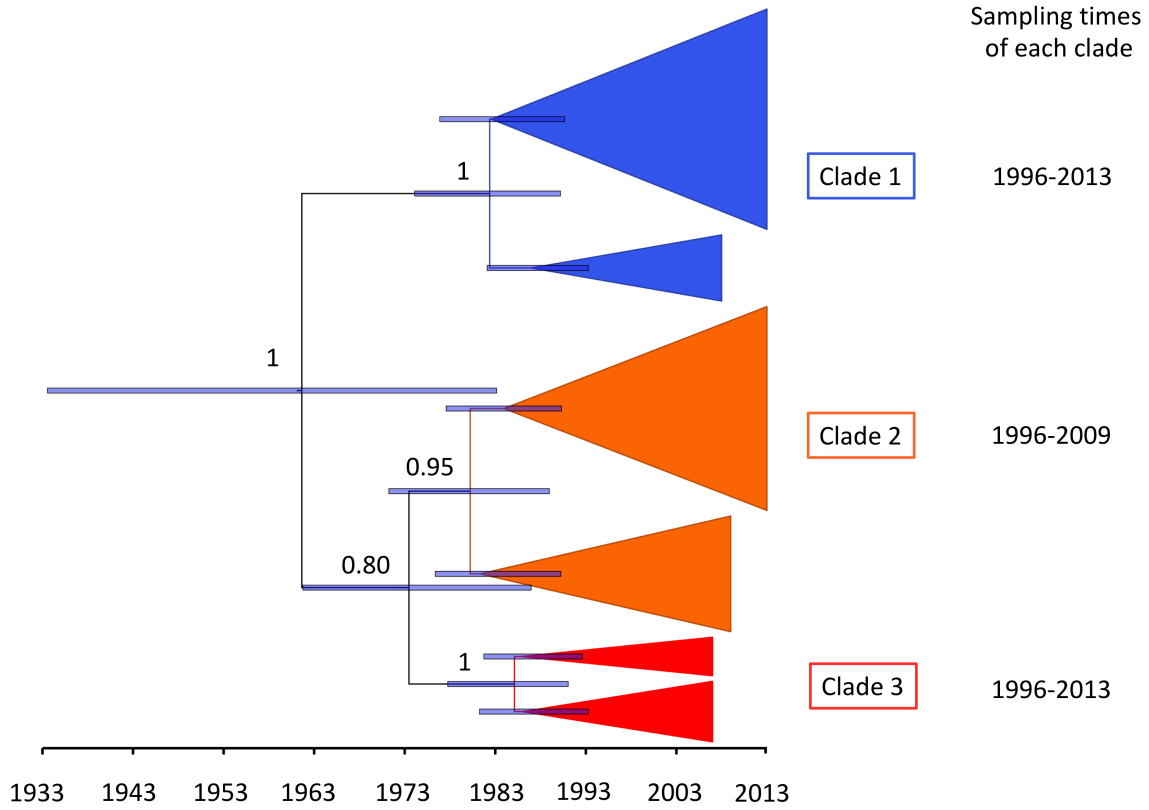
530 Table 1. Evidence of pathogen transition between host species. Results from a discrete ancestral  
531 trait mapping analysis.



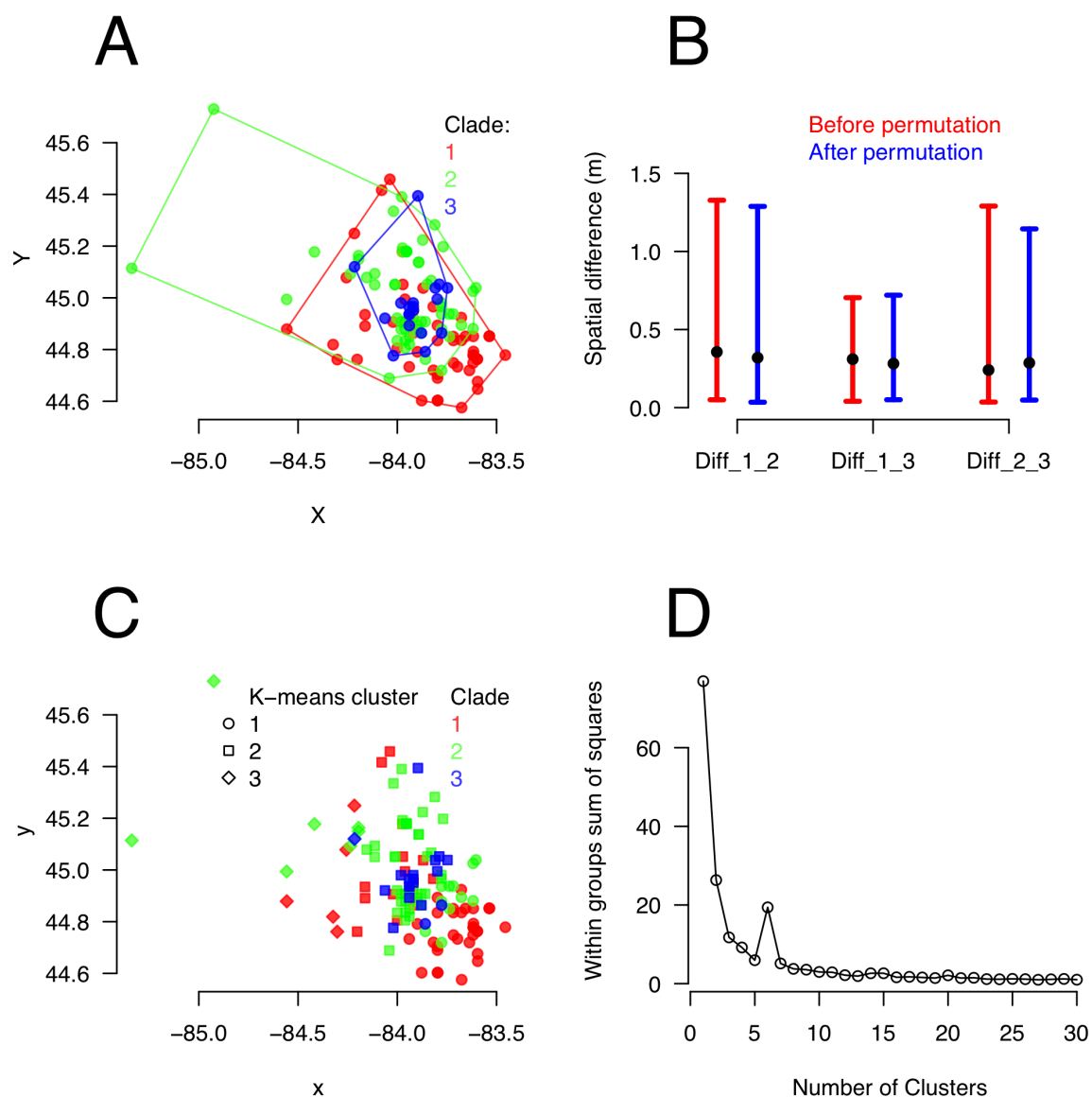
532  
533 Figure 1. Study area in northeastern Lower Peninsula of Michigan, USA with locations of  
534 bovine tuberculosis positive animals. Positive samples from deer that were spatially and  
535 temporally close to each positive elk and from the margins of the occupied elk range were  
536 selected for inclusion from among available archived isolates (39). This dataset was extended  
537 with 78 more positive deer samples randomly chosen from the available archived isolates.  
538 Positive cattle herds in the same area (9) were also selected together with trace backs of infected  
539 individuals from other herds (3). In total isolates from 5 elk (from 2000 to 2006), 117 deer  
540 (from 1996 to 2013) and 12 individual cattle (from 2000 to 2009) from 3 neighbouring herds



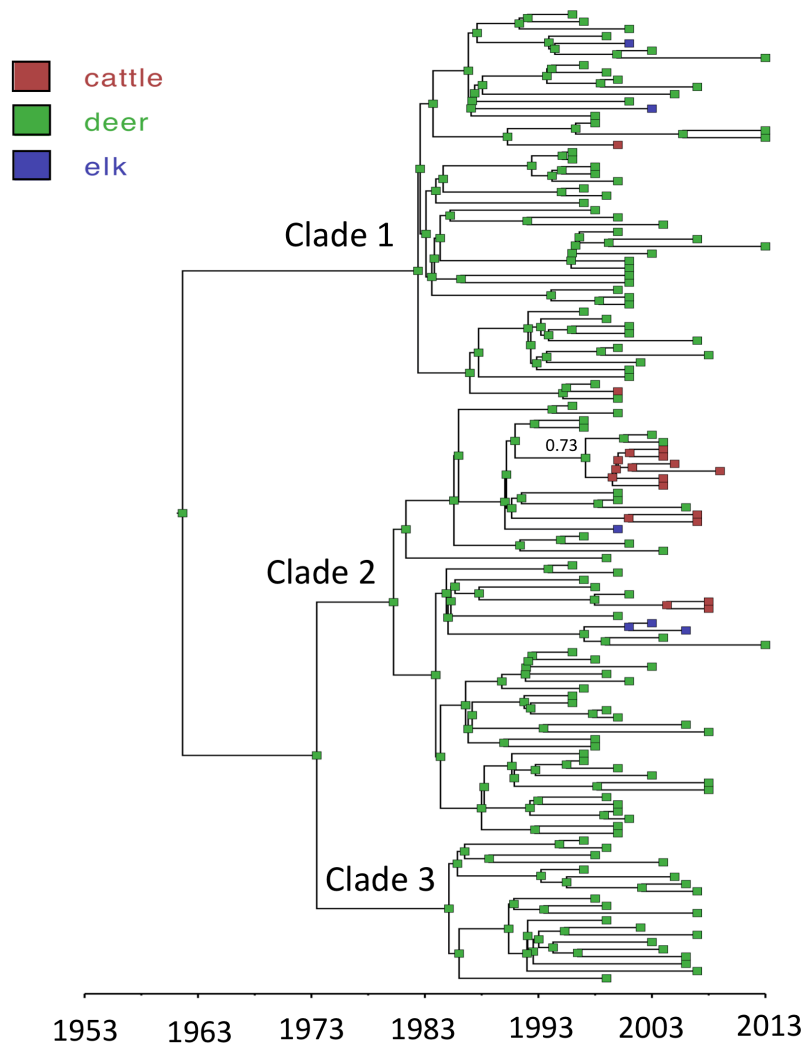
541 and 2 other herds identified by trace backs. The isolates were sampled from 8 counties:  
 542 Montmorency, Presque Isle, Otsego, Oscoda, Alpena, Alcona, Emmet and Antrim. Isolates that  
 543 were collected from the same host species in the same location are overlapped in the figure.  
 544  
 545



546  
 547  
 548 Figure 2. Time-calibrated maximum clade credibility tree of *Mycobacterium bovis* isolates.  
 549 Four *M. bovis* clades (C1-C3) were identified through Bayesian phylogenetic analyses of 117  
 550 *M. bovis* isolates sampled between 1996 and 2013 under an uncorrelated relaxed exponential  
 551 clock and extended skyline demographic model. Posterior support for major nodes is shown  
 552 with grey bars indicating the 95% highest posterior density intervals for node date estimates.

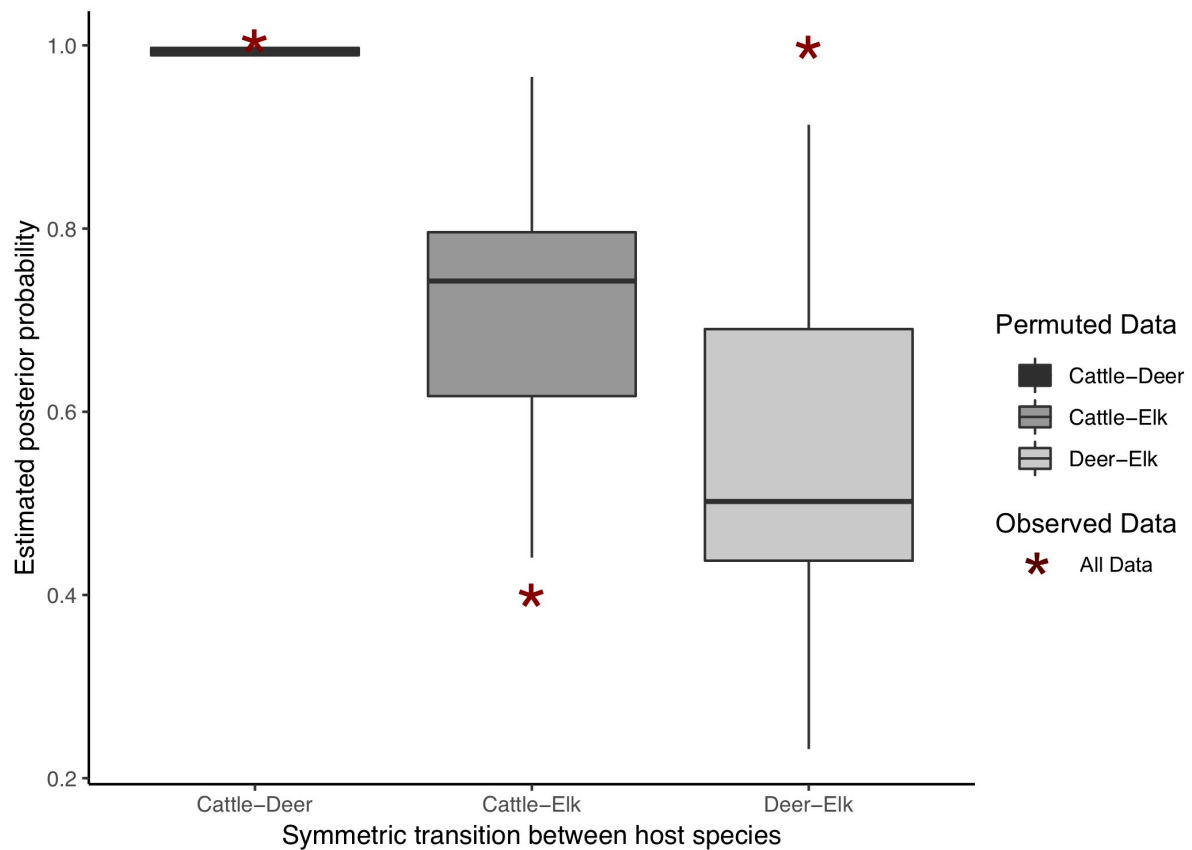


553  
 554 Figure 3. Spatial analysis of *Mycobacterium bovis* isolates. A. Spatial analysis of distribution  
 555 of *M. bovis* clades identified by Bayesian phylogenetic analysis. Each polygon represents the  
 556 minimum convex polygon of the sampled locations of the isolates of each clade. B.  
 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.  
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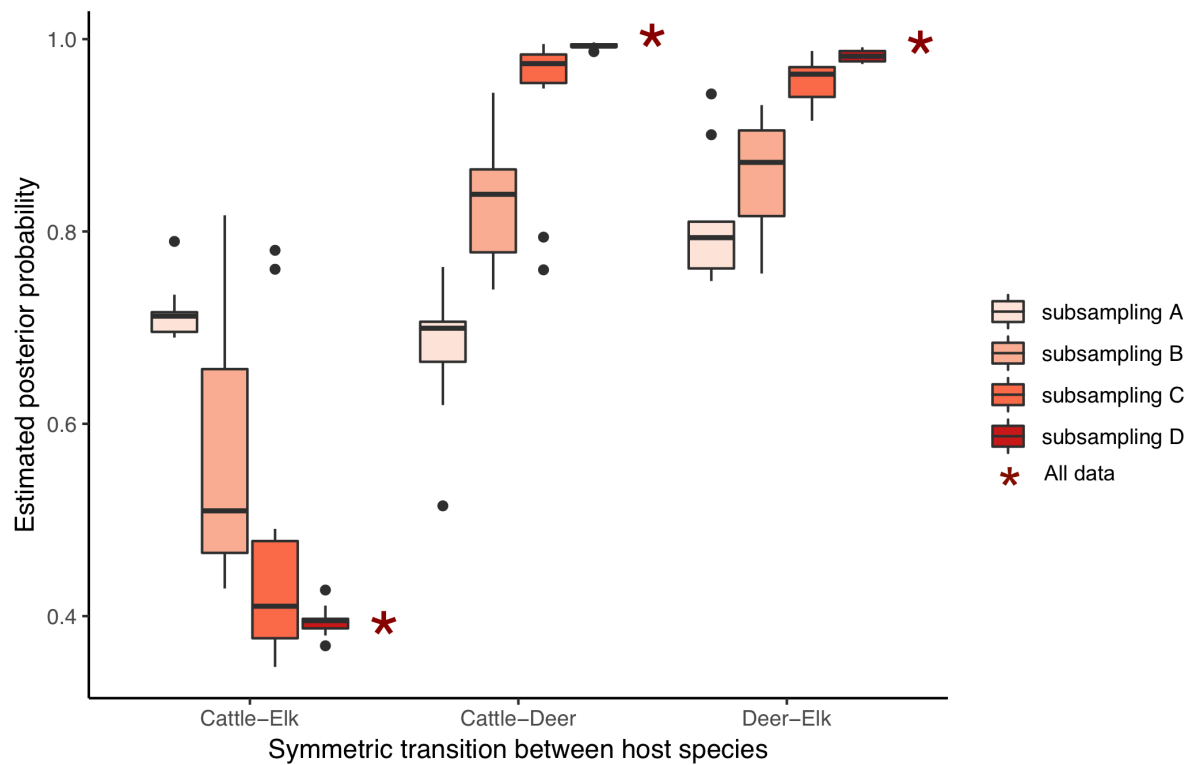
Figure 4. Ancestral host state reconstruction over the *Mycobacterium bovis* phylogeny. Maximum credibility tree was estimated under a model of symmetric host species transitions. Host state posterior probabilities (PP) are reported for ancestral nodes up to the most recent common ancestor. All nodes 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.



585  
 586 Figure 6. Comparison of the estimated posterior support of direct host species transition  
 587 between subsampled and observed data. The estimated posterior mean probability of each  
 588 interaction is the posterior probability that a particular transition rate is positive. If this  
 589 probability is high, then the data strongly support a model in which there is direct pathogen  
 590 transition between that particular pair of host species. The posterior means were estimated via  
 591 a Discrete Ancestral Trait Mapping performed in BEAST v2. The ‘Subsampled data’  
 592 correspond to three subsets of 10 files where the different isolates found in each species were  
 593 randomly chosen to be part of the new data set. Subsample A corresponds to isolates sampled  
 594 from five elk (‘Elk’), five randomly chosen cattle (‘Cattle’), and five randomly chosen deer  
 595 (‘Deer’); Subsample B corresponds to isolates sampled from five elk, nine cattle, and nine  
 596 randomly chosen deer; and Subsample C corresponds to isolates sampled from five elk, nine  
 597 cattle, and twenty four randomly chosen deer. The ‘All data’ correspond to the posterior mean  
 598 of each host species interaction output by one DATM analysis using all of the observed data,  
 599 which consists of five elk, twelve cattle, and 117 deer.

600 **REFERENCES**

- 601 Atwood, T C, T J Deliberto, H J Smith, J S Stevenson, and K C VerCauteren. 2009. “Spatial  
602 Ecology of Raccoons Related to Cattle and Bovine Tuberculosis in Northeastern  
603 Michigan.” *Journal of Wildlife Management* 73 (5): 647–54.
- 604 Auwera, G A Van der, M O Carneiro, C Hartl, R Poplin, G del Angel, A Levy-Moonshine, T  
605 Jordan, et al. 2013. “From FastQ Data to High-Confidence Variant Calls: The Genome  
606 Analysis Toolkit Best Practices Pipeline.” *Current Protocols in Bioinformatics*, no.  
607 SUPL.43. <https://doi.org/10.1002/0471250953.bi1110s43>.
- 608 Becker, Richard A., and Allan R. Wilks. 2016. “Maps: Draw Geographical Maps. R Package  
609 Version 3.1.1.” 2016. <https://cran.r-project.org/package=maps>.
- 610 Bengis, R G, R A Kock, and J Fischer. 2002. “Infectious Animal Diseases: The  
611 Wildlife/Livestock Interface.” *Revue Scientifique et Technique (International Office of  
612 Epizootics)* 21 (1): 53–65. <http://www.ncbi.nlm.nih.gov/pubmed/11974630>.
- 613 Berentsen, A. R., M. R. Dunbar, S. R. Johnson, S. Robbe-Austerman, L. Martinez, and R. L.  
614 Jones. 2011. “Active Use of Coyotes (*Canis Latrans*) to Detect Bovine Tuberculosis in  
615 Northeastern Michigan, USA.” *Veterinary Microbiology* 151 (1–2): 126–32.
- 616 Berentsen, Are R., Ryan S. Miller, Regina Misiewicz, Jennifer L. Malmberg, and Mike R.  
617 Dunbar. 2014. “Characteristics of White-Tailed Deer Visits to Cattle Farms:  
618 Implications for Disease Transmission at the Wildlife–Livestock Interface.” *European  
619 Journal of Wildlife Research* 60 (2). Springer Berlin Heidelberg: 161–70.  
620 <https://doi.org/10.1007/s10344-013-0760-5>.
- 621 Beyer, D. E. Jr. 1987. “Population and Habitat Management of Elk in Michigan.” Michigan  
622 State University, East Lansing, Michigan, USA.
- 623 Biek, R, A O’Hare, D Wright, T Mallon, C McCormick, R J Orton, S McDowell, H Trewby,  
624 R A Skuce, and R R Kao. 2012. “Whole Genome Sequencing Reveals Local  
625 Transmission Patterns of Mycobacterium Bovis in Sympatric Cattle and Badger  
626 Populations.” Edited by Oliver G. Pybus. *PLoS Pathogens* 8 (11). Public Library of  
627 Science: e1003008. <https://doi.org/10.1371/journal.ppat.1003008>.
- 628 Biek, R, O G Pybus, J O Lloyd-Smith, and X Didelot. 2015. *Measurably Evolving Pathogens  
629 in the Genomic Era. Trends in Ecology and Evolution*. Vol. 30.  
630 <https://doi.org/10.1016/j.tree.2015.03.009>.
- 631 Bird, Brian H., and Jonna A.K. Mazet. 2018. “Detection of Emerging Zoonotic Pathogens:  
632 An Integrated One Health Approach.” *Annual Review of Animal Biosciences* 6 (1).  
633 Annual Reviews : 121–39. <https://doi.org/10.1146/annurev-animal-030117-014628>.
- 634 Bivand, R, and N Lewin-Koh. 2017. “Maptools: Tools for Reading and Handling Spatial  
635 Objects. R Package Version 0.8-41.” 2017. <https://cran.r-project.org/package=maptools>.
- 636 Bivand, R, and C Rundel. 2017. “Rgeos: Interface to Geometry Engine - Open Source  
637 (GEOS). R Package Version 0.3-22.” 2017. <https://cran.r-project.org/package=rgeos>.
- 638 Bouckaert, R, J Heled, D Kühnert, T Vaughan, C H Wu, D Xie, M A Suchard, A Rambaut,  
639 and A J Drummond. 2014. “BEAST 2: A Software Platform for Bayesian Evolutionary  
640 Analysis.” Edited by Andreas Prlic. *PLoS Computational Biology* 10 (4). Public Library  
641 of Science: e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>.
- 642 Bruning-Fann, Colleen S., Stephen M. Schmitt, Scott D. Fitzgerald, Jean S. Fierke, Paul D.  
643 Friedrich, John B. Kaneene, Kathy A. Clarke, et al. 2001. “Bovine Tuberculosis in Free-  
644 Ranging Carnivores from Michigan.” *Journal of Wildlife Diseases* 37 (1). Wildlife  
645 Disease Association: 58–64. <https://doi.org/10.7589/0090-3558-37.1.58>.
- 646 Brunker, Kirstyn, Denise A Marston, Daniel L Horton, Sarah Cleaveland, Anthony R Fooks,  
647 Rudovick Kazwala, Chanasa Ngeleja, et al. 2015. “Elucidating the Phylodynamics of  
648 Endemic Rabies Virus in Eastern Africa Using Whole-Genome Sequencing.” *Virus  
649 Evolution* 1 (1). The Oxford University Press. <https://doi.org/10.1093/ve/vev011>.

- 650 Cowie, Catherine E., Michael R. Hutchings, Jose Angel Barasona, Christian Gortázar,  
651 Joaquín Vicente, and Piran C. L. White. 2016. “Interactions between Four Species in a  
652 Complex Wildlife: Livestock Disease Community: Implications for Mycobacterium  
653 Bovis Maintenance and Transmission.” *European Journal of Wildlife Research* 62 (1).  
654 Springer Berlin Heidelberg: 51–64. <https://doi.org/10.1007/s10344-015-0973-x>.
- 655 Crispell, J, R N Zadoks, S R Harris, B Paterson, D M Collins, G W De-Lisle, P Livingstone,  
656 et al. 2017. “Using Whole Genome Sequencing to Investigate Transmission in a Multi-  
657 Host System: Bovine Tuberculosis in New Zealand.” *BMC Genomics* 18 (180). BioMed  
658 Central. <https://doi.org/10.1186/s12864-017-3569-x>.
- 659 Darriba, D, G L Taboada, R Doallo, and D Posada. 2012. “JModelTest 2: More Models, New  
660 Heuristics and Parallel Computing.” *Nature Methods* 9 (8). Nature Research: 772–772.  
661 <https://doi.org/10.1038/nmeth.2109>.
- 662 Dehove, A, J Commault, M Petitclerc, M Teissier, and J Macé. 2012. “Economic Analysis  
663 and Costing of Animal Health: A Literature Review of Methods and Importance.” *Revue  
664 Scientifique et Technique (International Office of Epizootics)* 31 (2): 605–17, 591–604.  
665 <http://www.ncbi.nlm.nih.gov/pubmed/23413736>.
- 666 DePristo, M A, E Banks, R Poplin, K V Garimella, J R Maguire, C Hartl, A A Philippakis, et  
667 al. 2011. “A Framework for Variation Discovery and Genotyping Using Next-  
668 Generation DNA Sequencing Data.” *Nature Genetics* 43 (5): 491–98.  
669 <https://doi.org/10.1038/ng.806>.
- 670 Drummond, A J, S Y W Ho, M J Phillips, and A Rambaut. 2006. “Relaxed Phylogenetics and  
671 Dating with Confidence.” Edited by David Penny. *PLoS Biology* 4 (5). Public Library of  
672 Science: e88. <https://doi.org/10.1371/journal.pbio.0040088>.
- 673 Drummond, A J, G K Nicholls, A G Rodrigo, and W Solomon. 2002. “Estimating Mutation  
674 Parameters, Population History and Genealogy Simultaneously from Temporally Spaced  
675 Sequence Data.” *Genetics* 161 (3). Genetics Society of America: 1307–20.  
676 <https://doi.org/10.1006/tpbi.1999.1447>.
- 677 Drummond, A J, and A Rambaut. 2007. “BEAST: Bayesian Evolutionary Analysis by  
678 Sampling Trees.” *BMC Evolutionary Biology* 7 (1): 214. <https://doi.org/10.1186/1471-2148-7-214>.
- 680 Drummond, A J, A Rambaut, B Shapiro, and O G Pybus. 2005. “Bayesian Coalescent  
681 Inference of Past Population Dynamics from Molecular Sequences.” *Molecular Biology  
682 and Evolution* 22 (5). Oxford University Press: 1185–92.  
683 <https://doi.org/10.1093/molbev/msi103>.
- 684 Duchêne, S, D Duchêne, E C Holmes, and S Y W Ho. 2015. “The Performance of the Date-  
685 Randomization Test in Phylogenetic Analyses of Time-Structured Virus Data.”  
686 *Molecular Biology and Evolution* 32 (7). Oxford University Press: 1895–1906.  
687 <https://doi.org/10.1093/molbev/msv056>.
- 688 Duffy, S, and E C Holmes. 2009. “Validation of High Rates of Nucleotide Substitution in  
689 Geminiviruses: Phylogenetic Evidence from East African Cassava Mosaic Viruses.”  
690 *Journal of General Virology* 90 (6): 1539–47. <https://doi.org/10.1099/vir.0.009266-0>.
- 691 Fanning, A, and S Edwards. 1991. “Mycobacterium Bovis Infection in Human Beings in  
692 Contact with Elk (Cervus Elaphus) in Alberta, Canada.” *Lancet (London, England)* 338  
693 (8777): 1253–55.
- 694 Firth, C, A Kitchen, B Shapiro, M A Suchard, E C Holmes, and A Rambaut. 2010. “Using  
695 Time-Structured Data to Estimate Evolutionary Rates of Double-Stranded DNA  
696 Viruses.” *Molecular Biology and Evolution* 27 (9). Oxford University Press: 2038–51.  
697 <https://doi.org/10.1093/molbev/msq088>.
- 698 Fitzgerald, S D, and J B Kaneene. 2013. “Wildlife Reservoirs of Bovine Tuberculosis  
699 Worldwide: Hosts, Pathology, Surveillance, and Control.” *Veterinary Pathology* 50 (3).

700 SAGE Publications Sage CA: Los Angeles, CA: 488–99.  
701 <https://doi.org/10.1177/0300985812467472>.

702 Fitzgerald, Scott D., Laura S. Zwick, Kelly L. Diegel, Dale E. Berry, Steven V. Church,  
703 James G. Sikarskie, John B. Kaneene, and Willie M. Reed. 2003. “Experimental Aerosol  
704 Inoculation of Mycobacterium Bovis in North American Opossums (Didelphis  
705 Virginiana).” *Journal of Wildlife Diseases* 39 (2). Wildlife Disease Association : 418–  
706 23. <https://doi.org/10.7589/0090-3558-39.2.418>.

707 Gardy, J L, and N J Loman. 2018. “Towards a Genomics-Informed, Real-Time, Global  
708 Pathogen Surveillance System.” *Nature Reviews Genetics*.  
709 <https://doi.org/10.1038/nrg.2017.88>.

710 Gire, S K, A Goba, K G Andersen, R S G Sealfon, D J Park, L Kanneh, S Jalloh, et al. 2014.  
711 “Genomic Surveillance Elucidates Ebola Virus Origin and Transmission during the 2014  
712 Outbreak.” *Science*. <https://doi.org/10.1126/science.1259657>.

713 Gortázar, C, A Che Amat, and D J O’Brien. 2015. “Open Questions and Recent Advances in  
714 the Control of a Multi-Host Infectious Disease: Animal Tuberculosis.” *Mammal Review*  
715 45 (3). Wiley/Blackwell (10.1111): 160–75. <https://doi.org/10.1111/mam.12042>.

716 Gortazar, C, I Diez-Delgado, J A Barasona, J Vicente, J De La Fuente, and M Boadella. 2015.  
717 “The Wild Side of Disease Control at the Wildlife-Livestock-Human Interface: A  
718 Review.” *Frontiers in Veterinary Science* 1 (January). Frontiers: 27.  
719 <https://doi.org/10.3389/fvets.2014.00027>.

720 Grenfell, B T, O G Pybus, J R Gog, J L N Wood, J M Daly, J A Mumford, and E C Holmes.  
721 2004. “Unifying the Epidemiological and Evolutionary Dynamics of Pathogens.”  
722 *Science* 303 (5656): 327–32. <https://doi.org/10.1126/science.1090727>.

723 Hasegawa, M, H Kishino, and T Yano. 1985. “Dating the Human-Ape Split by a Molecular  
724 Clock of Mitochondrial DNA.” *Evolution* 22: 160–74.

725 Hassell, James M., Michael Begon, Melissa J. Ward, and Eric M. Fèvre. 2017. “Urbanization  
726 and Disease Emergence: Dynamics at the Wildlife–Livestock–Human Interface.” *Trends*  
727 *in Ecology & Evolution* 32 (1). Elsevier Current Trends: 55–67.  
728 <https://doi.org/10.1016/J.TREE.2016.09.012>.

729 Haydon, D T, S Cleaveland, L H Taylor, and M K Laurenson. 2002. “Identifying Reservoirs  
730 of Infection: A Conceptual and Practical Challenge.” *Emerging Infectious Diseases* 8  
731 (12): 1468–73. <https://doi.org/10.3201/eid0812.010317>.

732 Heled, Joseph, and Alexei J. Drummond. 2008. “Bayesian Inference of Population Size  
733 History from Multiple Loci.” *BMC Evolutionary Biology*. <https://doi.org/10.1186/1471-2148-8-289>.

734 ———. 2012. “Calibrated Tree Priors for Relaxed Phylogenetics and Divergence Time  
735 Estimation.” *Systematic Biology* 61 (1): 138–49. <https://doi.org/10.1093/sysbio/syr087>.

736 Hlokwe, T M, P van Helden, and A L Michel. 2014. “Evidence of Increasing Intra and Inter-  
737 Species Transmission of Mycobacterium Bovis in South Africa: Are We Losing the  
738 Battle?” *Preventive Veterinary Medicine* 115 (1–2): 10–17.  
739 <https://doi.org/10.1016/j.prevetmed.2014.03.011>.

740 Jones, B A, D Grace, R Kock, S Alonso, J Rushton, M Y Said, D McKeever, et al. 2013.  
741 “Zoonosis Emergence Linked to Agricultural Intensification and Environmental  
742 Change.” *Proceedings of the National Academy of Sciences* 110 (21). National Academy  
743 of Sciences: 8399–8404. <https://doi.org/10.1073/pnas.1208059110>.

744 Kamath, P L., J T. Foster, K P Drees, G Luikart, C Quance, N J Anderson, P R Clarke, et al.  
745 2016. “Genomics Reveals Historic and Contemporary Transmission Dynamics of a  
746 Bacterial Disease among Wildlife and Livestock.” *Nature Communications* 7 (May).  
747 Nature Research: 11448. <https://doi.org/10.1038/ncomms11448>.

748 Kao, R R, D T Haydon, S J Lycett, and P R Murcia. 2014. “Supersize Me: How Whole-



750 Genome Sequencing and Big Data Are Transforming Epidemiology.” *Trends in*  
751 *Microbiology* 22 (5): 282–91. <https://doi.org/10.1016/j.tim.2014.02.011>.

752 Kao, R R, M Price-Carter, and S Robbe-Austerman. 2016. “Use of Genomics to Track Bovine  
753 Tuberculosis Transmission.” *Revue Scientifique et Technique* 35 (1): 241–58.  
754 <https://doi.org/10.20506/rst.35.1.2430>.

755 Kass, R E, and A E Raftery. 1995. “Bayes Factors.” *Journal of the American Statistical*  
756 *Association*, 773–95.  
757 <https://www.stat.washington.edu/raftery/Research/PDF/kass1995.pdf>.

758 Kingman, J F C. 1982. “On the Genealogy of Large Populations.” *Journal of Applied*  
759 *Probability*. <https://doi.org/10.2307/3213548>.

760 Lartillot, N, and H Philippe. 2006. “Computing Bayes Factors Using Thermodynamic  
761 Integration.” Edited by Paul Lewis. *Systematic Biology* 55 (2): 195–207.  
762 <https://doi.org/10.1080/10635150500433722>.

763 Lavelle, M J, S L Kay, K M Pepin, D A Grear, H Campa, and K C VerCauteren. 2016.  
764 “Evaluating Wildlife-Cattle Contact Rates to Improve the Understanding of Dynamics of  
765 Bovine Tuberculosis Transmission in Michigan, USA.” *Preventive Veterinary Medicine*.  
766 <https://doi.org/10.1016/j.prevetmed.2016.10.009>.

767 Lemey, Philippe, Andrew Rambaut, Alexei J. Drummond, and Marc A. Suchard. 2009.  
768 “Bayesian Phylogeography Finds Its Roots.” Edited by Christophe Fraser. *PLoS*  
769 *Computational Biology* 5 (9). Public Library of Science: e1000520.  
770 <https://doi.org/10.1371/journal.pcbi.1000520>.

771 Li, H, and R Durbin. 2009. “Fast and Accurate Short Read Alignment with Burrows-Wheeler  
772 Transform.” *Bioinformatics* 25 (14): 1754–60.  
773 <https://doi.org/10.1093/bioinformatics/btp324>.

774 Livingstone, P G, G Nugent, G W de Lisle, and N Hancox. 2015. “Toward Eradication: The  
775 Effect of Mycobacterium Bovis Infection in Wildlife on the Evolution and Future  
776 Direction of Bovine Tuberculosis Management in New Zealand.” *New Zealand*  
777 *Veterinary Journal* 63 (1): 4–18. <https://doi.org/10.1080/00480169.2014.971082>.

778 Maio, N De, C H Wu, K M O’Reilly, and D Wilson. 2015. “New Routes to Phylogeography:  
779 A Bayesian Structured Coalescent Approximation.” *PLoS Genetics*.  
780 <https://doi.org/10.1371/journal.pgen.1005421>.

781 Manitoba Agriculture Department. n.d. “Timeline of Bovine Tuberculosis in Canadian and  
782 Manitoban Cattle and Bison.”

783 Mcbeth, M K, and E A Shanahan. 2004. “Public Opinion for Sale: The Role of Policy  
784 Marketers in Greater Yellowstone Policy Conflict.” *Policy Sciences*.  
785 <https://doi.org/10.1007/s11077-005-8876-4>.

786 McKenna, Aaron, Matthew Hanna, Eric Banks, Andrey Sivachenko, Kristian Cibulskis,  
787 Andrew Kernytsky, Kiran Garimella, et al. 2010. “The Genome Analysis Toolkit: A  
788 MapReduce Framework for Analyzing next-Generation DNA Sequencing Data.”  
789 *Genome Research* 20 (9): 1297–1303. <https://doi.org/10.1101/gr.107524.110>.

790 MDNR1. 2018. “Michigan Antlerless Deer Digest.” *Michigan Department of Natural*  
791 *Resources*, 2018. [mi.gov/deer](http://mi.gov/deer).

792 MDNR2. 2018. “Michigan Elk Digest.” *Michigan Department of Natural Resources*, 2018.  
793 [mi.gov/elk](http://mi.gov/elk).

794 MDNR3. n.d. “Michigan Elk Management Plan, Michigan Department of Natural  
795 Resources.”

796 Miller, R S, M L Farnsworth, and J L Malmberg. 2013. “Diseases at the Livestock–Wildlife  
797 Interface: Status, Challenges, and Opportunities in the United States.” *Preventive*  
798 *Veterinary Medicine* 110 (2). Elsevier: 119–32.  
799 <https://doi.org/10.1016/j.prevetmed.2012.11.021>.

800 Nugent, G, C Gortazar, and G Knowles. 2015. "The Epidemiology of Mycobacterium Bovis  
801 in Wild Deer and Feral Pigs and Their Roles in the Establishment and Spread of Bovine  
802 Tuberculosis in New Zealand Wildlife." *New Zealand Veterinary Journal* 63 (sup1): 54–  
803 67. <https://doi.org/10.1080/00480169.2014.963792>.

804 O'Brien, D J, M K Cosgrove, S M Schmitt, D J Yereb, E S Carlson, and M J Wilkins. 2004.  
805 "From the Field: An Occupational Safety Program for Wildlife Professionals Involved  
806 with Bovine Tuberculosis Surveillance." *Wildlife Society Bulletin* 32 (3): 992.

807 O'Brien, D J, S M Schmitt, D E Berry, S D Fitzgerald, T J Lyon, J R Vanneste, T M Cooley,  
808 S A Hogle, and J S Fierke. 2008. "Estimating the True Prevalence of Mycobacterium  
809 Bovis in Free-Ranging Elk in Michigan." *Journal of Wildlife Diseases* 44 (4). Wildlife  
810 Disease Association: 802–10. <https://doi.org/10.7589/0090-3558-44.4.802>.

811 O'Brien, D J, S M Schmitt, J S Fierke, S A Hogle, S R Winterstein, T M Cooley, W E Moritz,  
812 et al. 2002. "Epidemiology of Mycobacterium Bovis in Free-Ranging White-Tailed  
813 Deer, Michigan, USA, 1995-2000." *Preventive Veterinary Medicine* 54 (1): 47–63.  
814 [https://doi.org/10.1016/S0167-5877\(02\)00010-7](https://doi.org/10.1016/S0167-5877(02)00010-7).

815 O'Brien, D J, S M Schmitt, S D Fitzgerald, and D E Berry. 2011. "Management of Bovine  
816 Tuberculosis in Michigan Wildlife: Current Status and near Term Prospects." *Veterinary  
817 Microbiology* 151 (1–2): 179–87. <https://doi.org/10.1016/j.vetmic.2011.02.042>.

818 O'Brien, D J, S M Schmitt, S D Fitzgerald, D E Berry, and G J Hickling. 2006. "Managing  
819 the Wildlife Reservoir of Mycobacterium Bovis: The Michigan, USA, Experience." In  
820 *Veterinary Microbiology*, 112:313–23. <https://doi.org/10.1016/j.vetmic.2005.11.014>.

821 O'Brien, D J, S M Schmitt, and D Jessup. 2014. "From Wildlife to Livestock--and Vice  
822 Versa: Disease Transmission Creates a Thorny Wildlife-Livestock Divide." *The Wildlife  
823 Professional* 8: 40–43.

824 Palmer, M V. 2013. "Mycobacterium Bovis: Characteristics of Wildlife Reservoir Hosts." *Transboundary and Emerging Diseases*. <https://doi.org/10.1111/tbed.12115>.

825 Palmer, M V, W R Waters, and D L Whipple. 2002. "Susceptibility of Raccoons ( OF  
826 RACCOONS (Procyon Lotor) to Infection with Mycobacterium Bovis." *Journal of  
827 Wildlife Diseases* 38 (2). Wildlife Disease Association: 266–74.  
828 <https://doi.org/10.7589/0090-3558-38.2.266>.

829 Paradis, E, J Claude, and K Strimmer. 2004. "APE: Analyses of Phylogenetics and Evolution  
830 in R Language." *Bioinformatics* 20 (2): 289–90.  
831 <http://www.ncbi.nlm.nih.gov/pubmed/14734327>.

832 Parish, T, and N G Stocker. 2002. *Mycobacterium Tuberculosis Protocols. Methods in  
833 Molecular Medicine*. <https://doi.org/10.1385/1592591477>.

834 Pybus, O G, and A Rambaut. 2009. "Evolutionary Analysis of the Dynamics of Viral  
835 Infectious Disease." *Nature Reviews Genetics*. <https://doi.org/10.1038/nrg2583>.

836 Rambaut, A, T Lam, L Carvalho, and O G Pybus. 2016. "Exploring the Temporal Structure of  
837 Heterochronous Sequences Using TempEst (Formerly Path-O-Gen)." *Virus Evolution* 2  
838 (1): vew007. <https://doi.org/10.1093/ve/vew007>.

839 Rambaut, A, M A Suchard, D Xie, and A J Drummond. 2014. "Tracer v1.6." 2014.  
840 <http://beast.bio.ed.ac.uk/Tracer>.

841 Ramsden, C., E. C. Holmes, and M. A. Charleston. 2008. "Hantavirus Evolution in Relation  
842 to Its Rodent and Insectivore Hosts: No Evidence for Codivergence." *Molecular Biology  
843 and Evolution* 26 (1): 143–53. <https://doi.org/10.1093/molbev/msn234>.

844 RCoreTeam. 2014. "R Core Team. R: A Language and Environment for Statistical  
845 Computing. R Foundation for Statistical Computing." R Foundation for Statistical  
846 Computing, Vienna Austria. 2014. <https://www.r-project.org/>.

847 Rhyan, J C, D A Saari, E S Williams, M W Miller, A J Davis, and A J Wilson. 1992. "Gross  
848 and Microscopic Lesions of Naturally-Occurring Tuberculosis in a Captive Herd of  
849

850 Wapiti (*Cervus-Elaphus-Nelsoni*) in Colorado.” *Journal of Veterinary Diagnostic*  
851 *Investigation* 4 (4): 428–33. <https://doi.org/10.1177/104063879200400411>.

852 Ruhl, J D. 1984. “Elk Movements and Habitat Utilization in Northern Michigan.” Michigan  
853 State University, Lansing, Michigan, USA.

854 Schmitt, S M, S D Fitzgerald, T M Cooley, C S Bruning-Fann, L Sullivan, D Berry, T  
855 Carlson, R B Minnis, J B Payeur, and J Sikarskie. 1997. “Bovine Tuberculosis in Free-  
856 Ranging White-Tailed Deer from Michigan.” *Journal of Wildlife Diseases* 33 (4): 749–  
857 58. <https://doi.org/10.7589/0090-3558-33.4.749>.

858 Shury, T K. 2015. “The Epidemiology of Bovine Tuberculosis (*Mycobacterium Bovis*) in the  
859 Greater Riding Mountain Ecosystem.” University of Saskatchewan.

860 Shury, T K, and D Bergeson. 2011. “Lesion Distribution and Epidemiology of  
861 *Mycobacterium Bovis* in Elk and White-Tailed Deer in South-Western Manitoba,  
862 Canada.” *Veterinary Medicine International* 2011 (June). Hindawi: 591980.  
863 <https://doi.org/10.4061/2011/591980>.

864 Skuce, R A, A R Allen, W J McDowell, and S W J McDowell. 2012. “Herd-Level Risk  
865 Factors for Bovine Tuberculosis: A Literature Review.” *Veterinary Medicine*  
866 *International* 2012 (621210): 10. <https://doi.org/10.1155/2012/621210>.

867 Tavare, S. 1986. “Some Probabilistic and Statistical Problems in the Analysis of DNA  
868 Sequences.” *Lectures on Mathematics in the Life Sciences*. [https://doi.org/citeulike-](https://doi.org/citeulike-article-id:4801403)  
869 [article-id:4801403](https://doi.org/citeulike-article-id:4801403).

870 Trewby, H, D Wright, E L Breadon, S J Lycett, T R Mallon, C McCormick, P Johnson, et al.  
871 2016. “Use of Bacterial Whole-Genome Sequencing to Investigate Local Persistence and  
872 Spread in Bovine Tuberculosis.” *Epidemics* 14 (March): 26–35.  
873 <https://doi.org/10.1016/j.epidem.2015.08.003>.

874 Tsao, K, S Robbe-Austerman, R S Miller, K Portacci, D A Grear, and C Webb. 2014.  
875 “Sources of Bovine Tuberculosis in the United States.” *Infection, Genetics and Evolution*  
876 28: 137–43. <https://doi.org/10.1016/j.meegid.2014.09.025>.

877 Tullar, B F J. 1983. “A Long-Lived White-Tailed Deer.” *New York Fish and Game Journal*  
878 30 (119).

879 Volz, E M, K Koelle, and T Bedford. 2013. “Viral Phylodynamics.” *PLoS Computational*  
880 *Biology*. <https://doi.org/10.1371/journal.pcbi.1002947>.

881 Volz, E M, S L Kosakovsky Pond, M J Ward, A J Leigh Brown, and Simon D W Frost. 2009.  
882 “Phylodynamics of Infectious Disease Epidemics.” *Genetics*.  
883 <https://doi.org/10.1534/genetics.109.106021>.

884 Walsh, Daniel Paul. 2007. “Population Estimation and Fixed Kernel Analyses of Elk in  
885 Michigan.” Michigan State University.

886 Walter, W D, J W Fischer, C W Anderson, D R Marks, T Deliberto, S Robbe-Austerman, and  
887 K C Vercauteren. 2013. “Surveillance and Movements of Virginia Opossum (*Didelphis*  
888 *Virginiana*) in the Bovine Tuberculosis Region of Michigan.” *Epidemiology and*  
889 *Infection* 141 (7). Cambridge University Press: 1498–1508.  
890 <https://doi.org/10.1017/S0950268813000629>.

891 Welburn, S. 2011. “One Health: The 21st Century Challenge.” *The Veterinary Record* 168  
892 (23). British Medical Journal Publishing Group: 614–15.  
893 <https://doi.org/10.1136/vr.d3528>.

894 Wiethoelter, A K, D Beltrán-Alcrudo, R Kock, and S M Mor. 2015. “Global Trends in  
895 Infectious Diseases at the Wildlife-Livestock Interface.” *Proceedings of the National*  
896 *Academy of Sciences of the United States of America* 112 (31): 9662–67.  
897 <https://doi.org/10.1073/pnas.1422741112>.

898  
899

900