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

Abstract (250)

The role of wildlife in the persistence and spread of livestock diseases is difficult to quantify and control. These difficulties are exacerbated when several wildlife species are potentially involved. Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, has experienced an ecological shift in Michigan, with spillover from cattle leading to an endemically infected white-tailed deer (deer) population. It has potentially substantial implications for the health and well-being of both wildlife and livestock and incurs a significant economic cost to industry and government. Deer are known to act as a reservoir of infection, with evidence of *M. bovis* transmission to sympatric elk and cattle populations. However, the role of elk in the circulation of *M. bovis* is uncertain – they are few in number, but range further than deer, so may enable long distance spread. Combining Whole Genome Sequences (WGS) for *M. bovis* isolates from exceptionally well-observed populations of elk, deer and cattle with spatio-temporal locations, we use spatial and Bayesian phylogenetic analyses to show strong spatio-temporal admixture of *M. bovis* isolates. Clustering of bTB in elk and cattle suggests either intraspecies transmission within the two populations, or exposure to a common source. However, there is no support for significant pathogen transfer amongst elk and cattle, and our data are in accordance with existing evidence that interspecies transmission in Michigan is likely only maintained by deer. This study demonstrates the value of whole-genome population studies of *M. bovis* transmission at the wildlife-livestock interface, providing insights into bTB management in an endemic system.

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

relationships among the host states at the branch tips (from the sampled isolates), and has provided, for example, evidence that free-ranging elk are currently a self-sustaining brucellosis reservoir and the source of livestock infections in the Great Yellowstone Ecosystem (Kamath et al. 2016).

Control of infectious diseases at the wildlife-livestock interface

Infectious diseases at the wildlife-livestock interface endanger the health and well-being of wildlife and livestock. They contribute to considerable economic losses to each sector, including wildlife-related sectors such as hunting and wildlife tourism, and they also represent a potential burden to the whole ecosystem (Wiethoelter et al. 2015; Hassell et al. 2017). The livestock sector is affected through increased mortality and reduced livestock productivity, as well as indirect losses associated with cost of surveillance, decreased market values, food insecurity, and impacts on farmers' livelihood (Dehove et al. 2012).The recreational manipulation of the natural environment to increase the density of wildlife beyond its normal carrying capacity, together with agricultural intensification and deforestation, have resulted in interactions between wildlife and livestock becoming more frequent (Jones et al. 2013; Are R. Berentsen et al. 2014; Lavelle et al. 2016; Skuce et al. 2012; Cowie et al. 2016), creating a dynamic and bidirectional opportunity for pathogens to circulate freely within and across species (Bengis, Kock, and Fischer 2002), via direct and/or indirect routes (use of communal environment, shared resources, etc). The control of infectious diseases at the wildlife-livestock interface is particularly challenging because of the differences in disease control efforts aimed respectively at both livestock and wildlife populations (Gortazar et al. 2015; Bird and Mazet 2018), as these are usually managed by different organisational entities (Miller, Farnsworth, and Malmberg 2013; Welburn 2011; Mcbeth and Shanahan 2004).

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

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

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 (≥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 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

elk analyses. Figure 5 shows that the estimated interactions between deer and elk, and cattle and elk with the real data (presented by stars) differ from the ones estimated with the randomized data sets (presented by boxplots), with the exception of the estimated interactions between cattle and deer from the real and random data sets, which overlap with each other. This overlap suggests that pathogen migration between these two species is consistent with it being a random process. If this is the case, then increases of bTB in cattle may simply be attributable to increases in deer population densities and infection levels. The sensitivity analyses to show the effect of sample size (of each host species) on the interspecies interaction, show that this measure only influences our results under extreme down-sampling (dataset A). However, with lesser but still substantial down-sampling of data (B, C and D; which have variations in sample size for each host species), our analyses show a similar pattern to the original data: strong support for interactions between deer and cattle and deer and elk, and low support for interactions between cattle and elk (Figure 6, Table 1). Finally, the addition of 1 or 2 "elk" samples to our pool of isolates (by replacing deer isolates) were shown to be insufficient to change our results (Figure S7).

Discussion

This analysis is one of the few genomic studies examining bacterial transmission at the wildlife-livestock interface (Kamath et al. 2016) in the United States and highlights the important role that genomics and phylodynamic approaches play in improving our understanding of fine scale transmission patterns. Using *M. bovis* genomic data from different host species with a time frame of 17 years, we showed that, even with a slow, highly variable substitution rate, WGS has remarkable power to identify the likely roles of different host species in the transmission dynamics of endemically circulating diseases, independent of other epidemiological evidence. 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*

virginiana) have been shown to be bTB spillovers in this area (Bruning-Fann et al. 2001; Walter et al. 2013; A. R. Berentsen et al. 2011). It could be though direct contact (however unlikely (Scott D. Fitzgerald et al. 2003)), or through environment contamination. Both raccoons and opossums are found to share communal dens resulting in increased interaction when resources are abundant such as around feed stockpiled for livestock (Palmer, Waters, and Whipple 2002; Atwood et al. 2009), and when they have a chance, they use the same stored feed, water sources, and feed being consumed by cattle (Bruning-Fann et al. 2001; Atwood et al. 2009; Walter et al. 2013), increasing the chances of contamination. More studies on these populations would help to understand their contribution to the spread of bTB.

In Michigan, bTB has been a concern of management by both wildlife and agriculture agencies for two decades. Prospects for eradication are uncertain, and the ongoing costs of disease management necessitate the use of innovative methods to inform management decisions. By providing insights into reservoir status and the likelihood of interspecies transmission, genomic analyses such as this supplement traditional epidemiologic and pathologic data, advancing efficient and effective use of both bTB surveillance and control resources.

Data Accessibility

The raw sequence files (FASTQ) were submitted to the NCBI Sequence Read Archive under the Bioproject accession number: PRJNA251692. The individual isolates can be accessed under the following Biosample accession numbers: SAMN07339977 - SAMN07340029 and SAMN10254813 – SAMN10254893. Information about metadata associated to each isolate is in Table S3. The R scripts used for this publication are freely available on the following Github link: https://github.com/lsalvador/WGS_Michigan_Project.

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.

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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)
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.

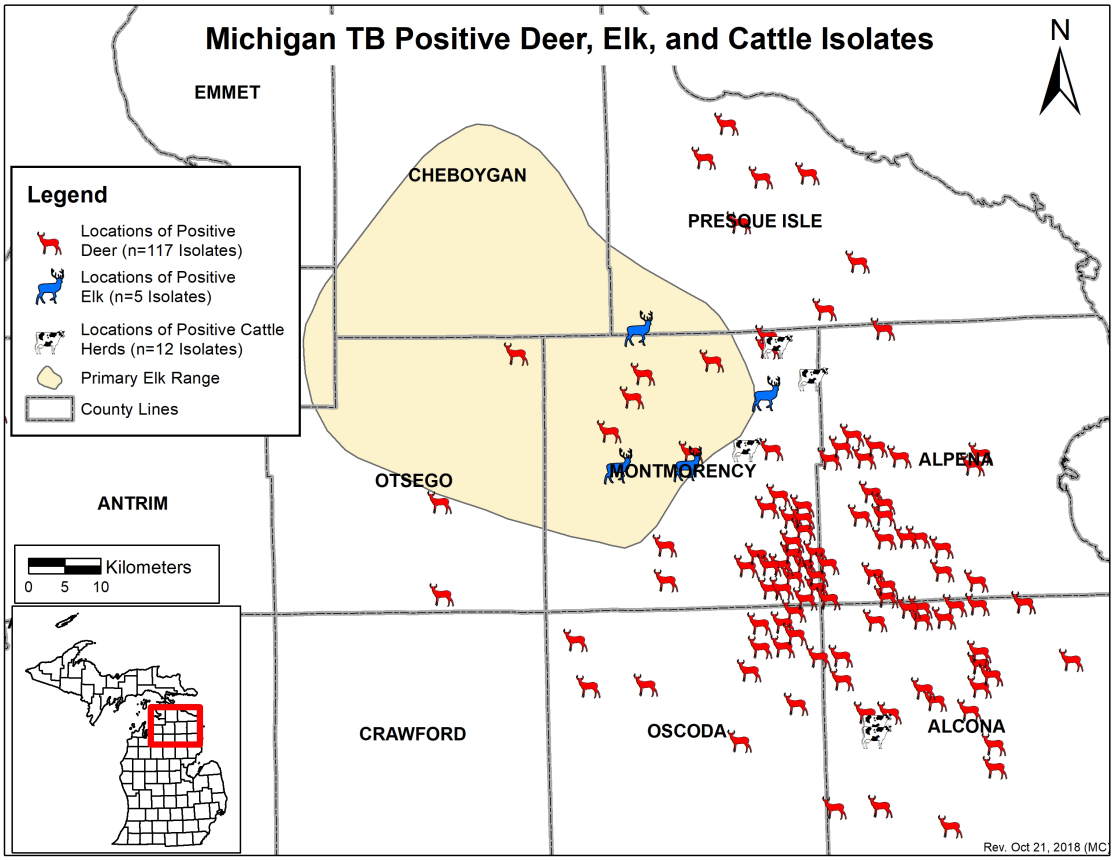
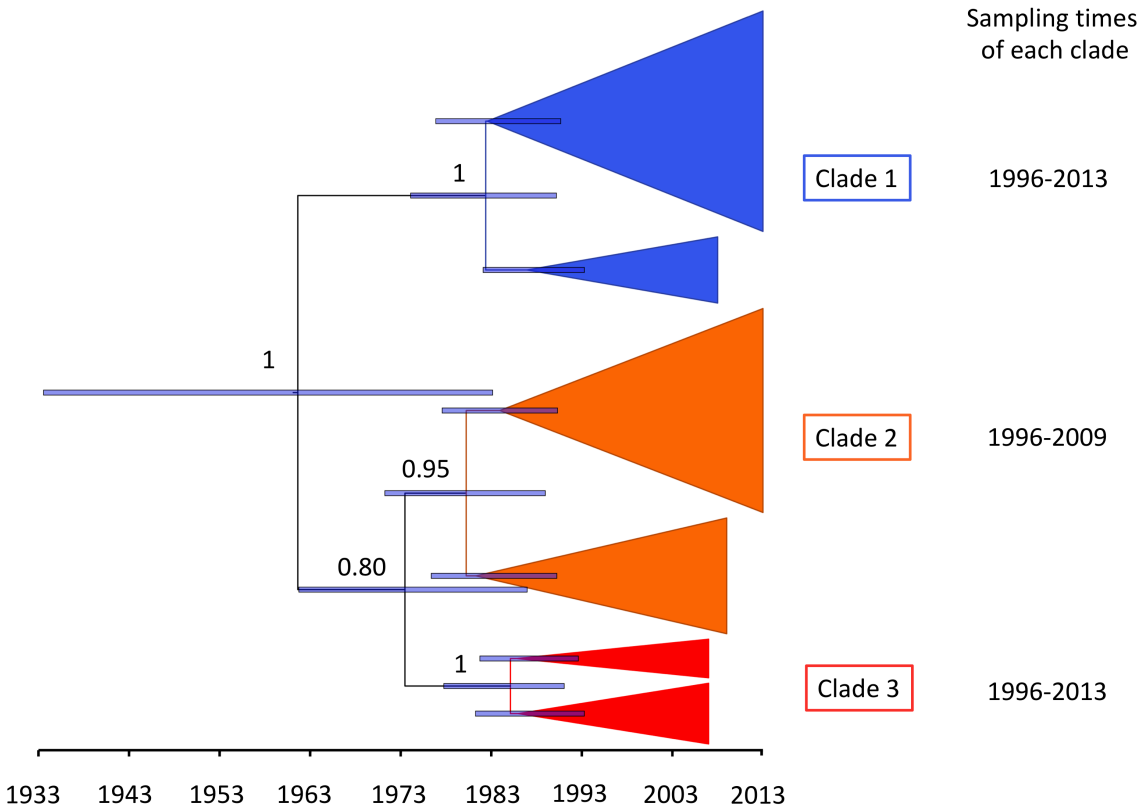


Figure 1. Study area in northeastern Lower Peninsula of Michigan, USA with locations of bovine tuberculosis positive animals. Positive samples from deer that were spatially and temporally close to each positive elk and from the margins of the occupied elk range were selected for inclusion from among 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

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.

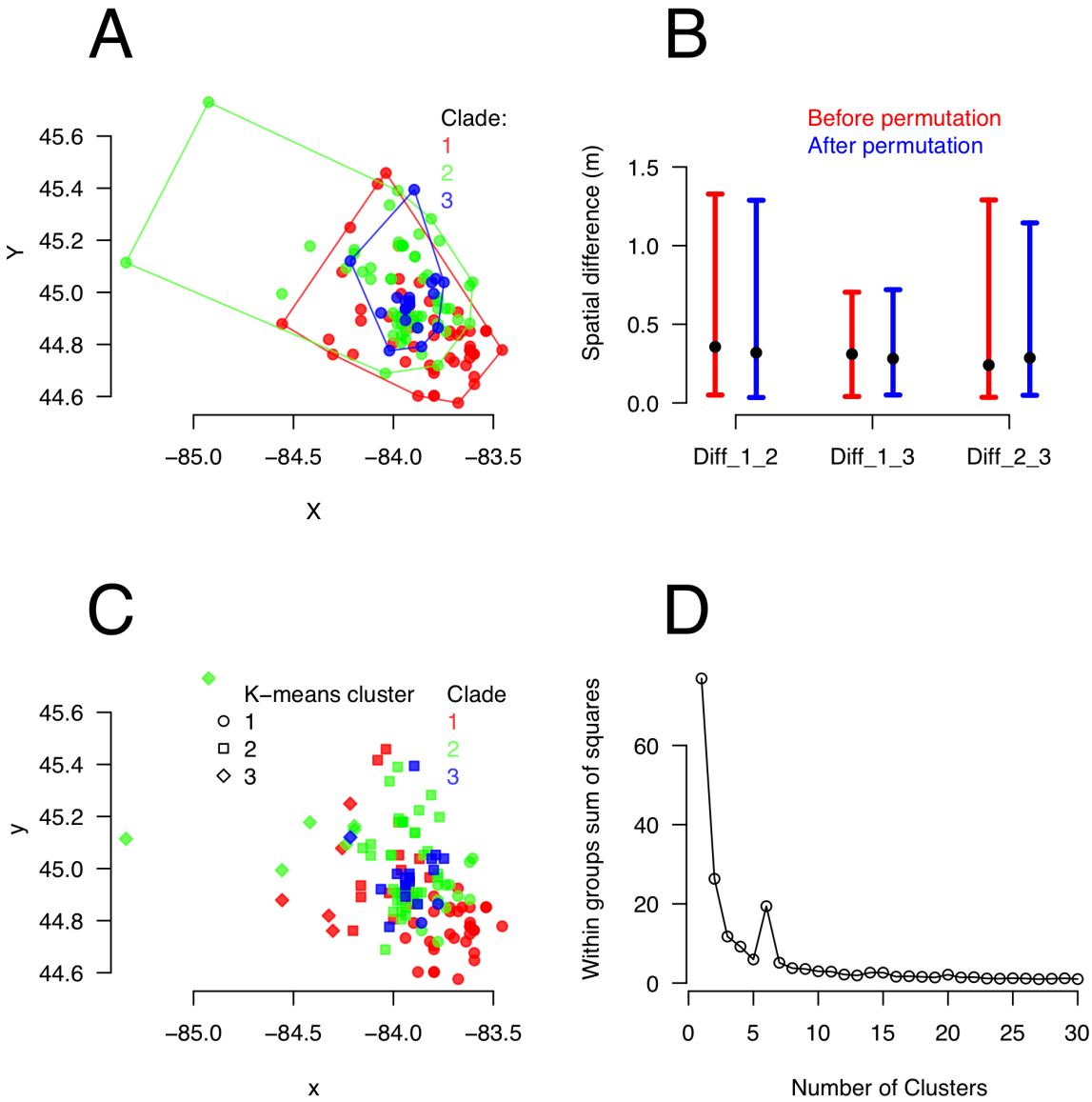


Figure 3. Spatial analysis of *Mycobacterium bovis* isolates. A. Spatial analysis of distribution of *M. bovis* clades identified by Bayesian phylogenetic analysis. Each polygon represents the minimum convex polygon of the sampled locations of the isolates of each clade. B. Comparison of spatial distances between estimated and permuted clades. For every pair of clades being compared we have randomly selected 1000 isolates from each. For each random pair of isolates we calculated the spatial distance between them. This analysis was repeated with random (permuted) clade assignments. C. K-means analysis with 3 clusters (represented by symbols) versus 3 clades (represented by colors). D. Optimal number of clusters estimated by within group sum of squares (distances between individuals within each cluster). The optimal number of clusters will be the number after which within cluster differences become minimal; here this occurs after ~ 3 clusters.

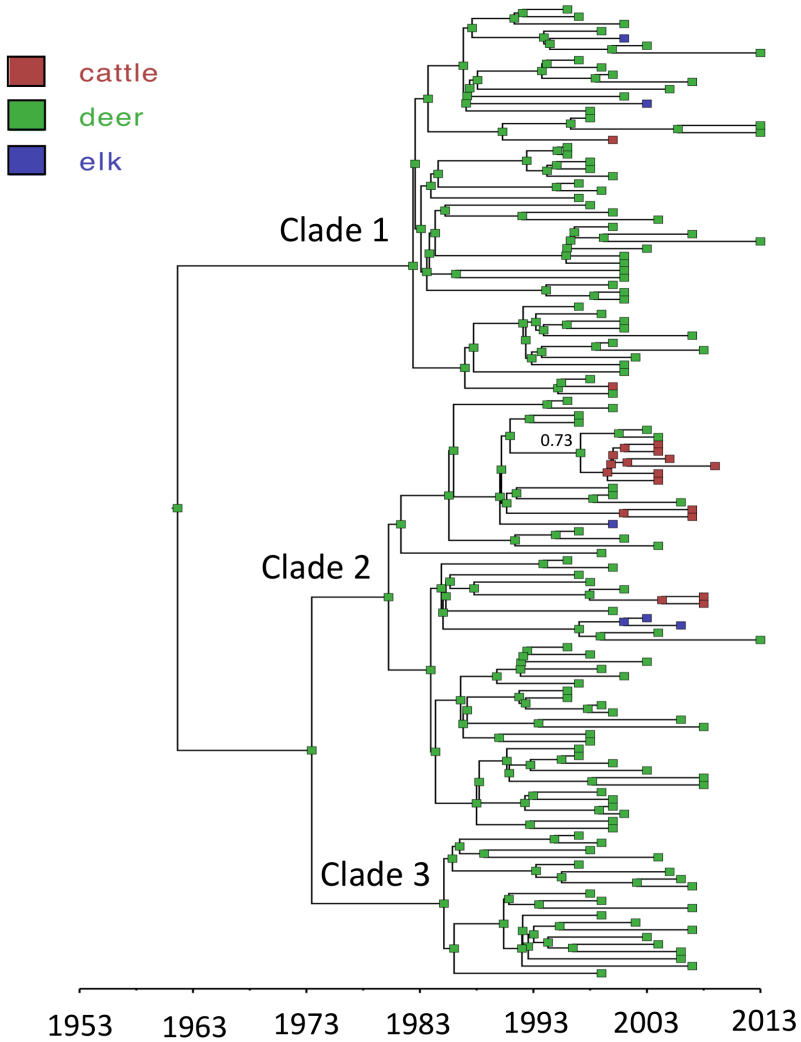


Figure 4. Ancestral host state reconstruction over the *Mycobacterium bovis* phylogeny. Maximum credibility tree was estimated under a model of symmetric host species transitions. Host state posterior probabilities (PP) are reported for ancestral nodes up to the most recent common ancestor. All nodes 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).

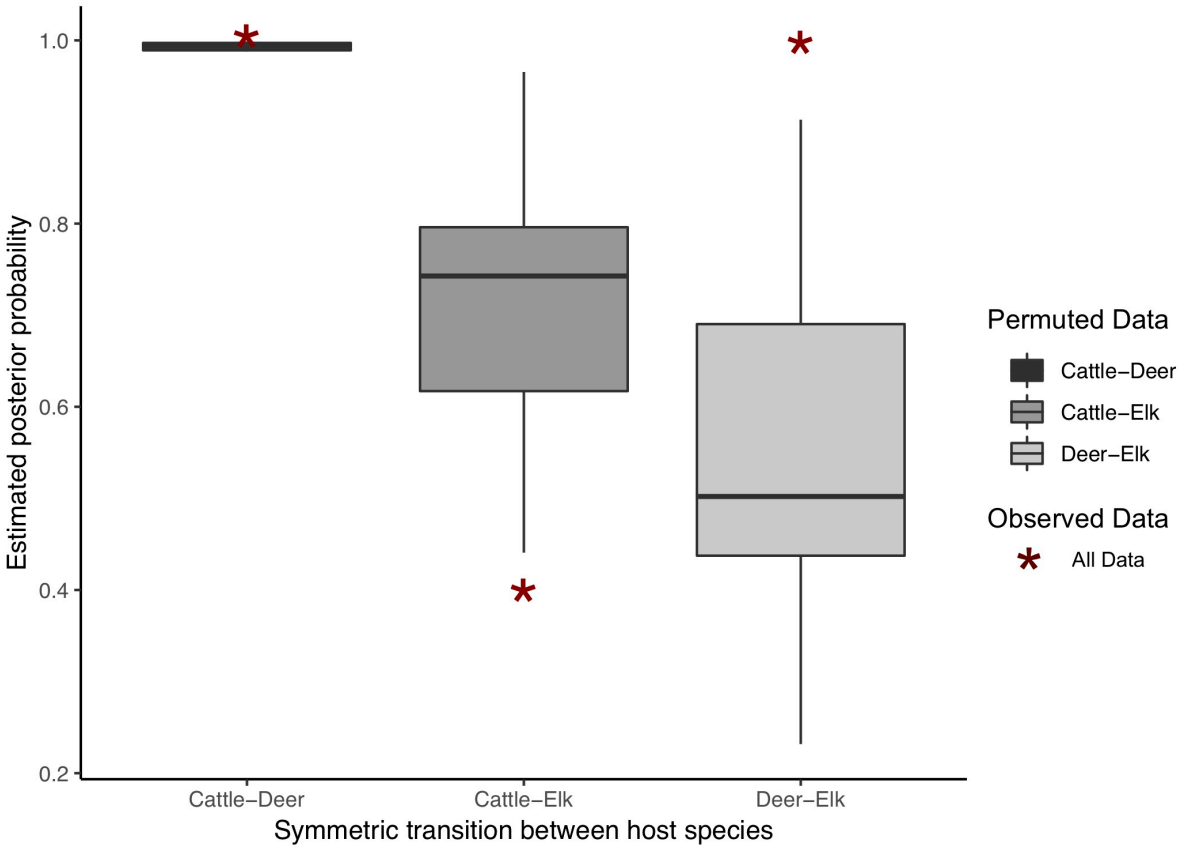


Figure 5. Comparison of the estimated posterior support of direct host species transition between permuted and observed data. The estimated posterior mean probability of each host species interaction is the posterior probability that a particular transition rate is positive. If this probability is high, 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 'Permuted data' correspond to the posterior means of 10 BEAST runs of each interaction after permuting the host species labels each time. The 'Observed data' correspond to the posterior mean of each interaction using the observed data.

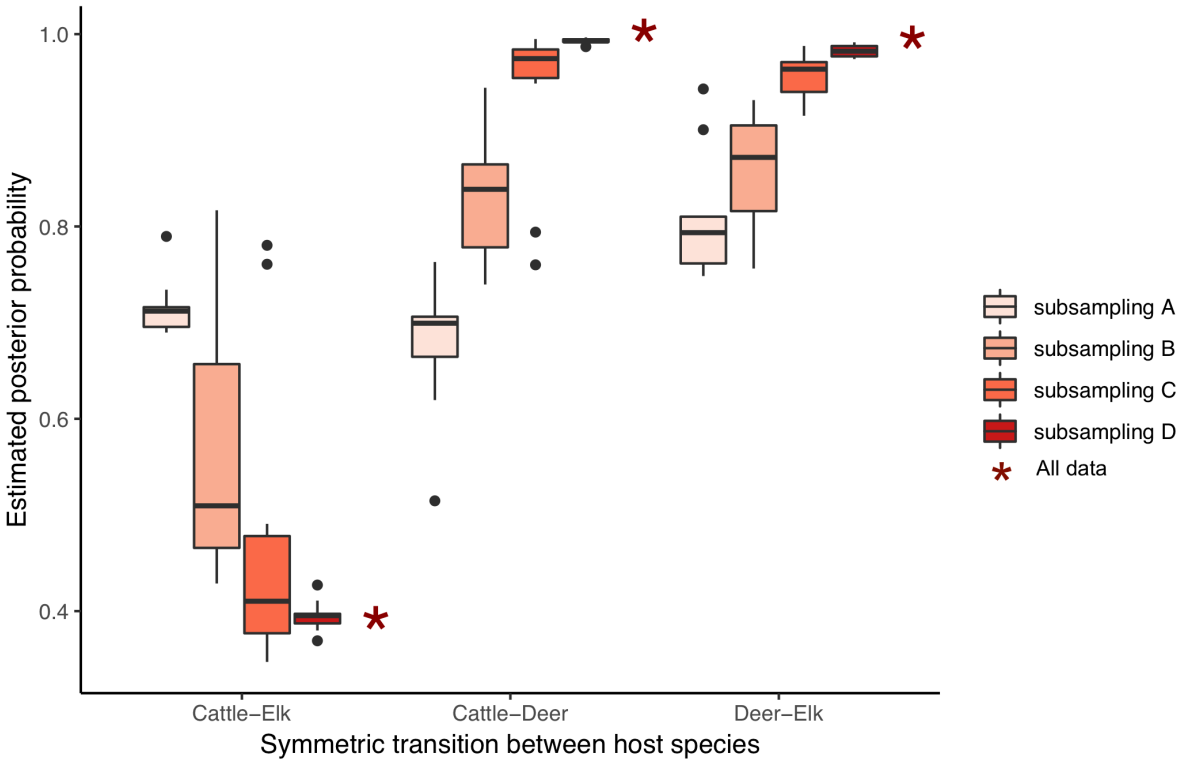


Figure 6. Comparison of the estimated posterior support of direct host species transition between subsampled and observed data. The estimated posterior mean probability of each interaction is the posterior probability that a particular transition rate is positive. If this probability is high, then the 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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