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Sources of bovine tuberculosis in the United States

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ABSTRACT

Despite control and eradication efforts, bovine tuberculosis continues to be identified at low levels among cattle in the United States. We evaluated possible external sources of infection by characterizing the genetic relatedness of bovine tuberculosis from a national database of reported infections, comparing strains circulating among US cattle with those of imported cattle, and farmed and wild cervids.

Farmed cervids maintained a genetically distinct *Mycobacterium bovis* strain, and cattle occasionally became infected with this strain. In contrast, wild cervids acted as an epidemiologically distinct group, instead hosting many of the same strains found in cattle, and the data did not show a clear transmission direction. Cattle from Mexico hosted a higher overall richness of strains than US cattle, and many of those strains were found in both US and Mexican cattle. However, these two populations appeared to be well-mixed with respect to their *M. bovis* lineages, and higher resolution data is necessary to infer the direction of recent transmission.

Overall patterns of both host and geographic distributions were highly variable among strains, suggesting that different sources or transmission mechanisms are contributing to maintaining different strains.

1. Introduction

1.1. Epidemiology and evolution

Mycobacterium bovis, the causative agent of bovine tuberculosis (bTB), continues to infect cattle in the United States at a low level despite control and eradication efforts. Routes of transmission include shared feed, other environmental fomites, and direct contact. There are multiple possible recent infection sources of *M. bovis* transmission to US cattle, including: (1) infected cervids (deer and their relatives), and (2) cattle imported from bTB-endemic areas outside the US. Here we consider evidence for these potential sources in the context of *M. bovis* genetic relatedness.

Our inferences rely on relationships among strains, which we evaluate by reconstructing clonal complexes. The evolutionary model of clonal expansion that gives rise to these complexes is well supported in previous *M. bovis* population genetic studies (Gutiérrez Reyes et al., 2012; Smith, 2012; Smith et al., 2003, 2006). In this model, a founder strain spreads among many individuals, and as it reproduces and is transmitted, subsequent mutations produce a group of closely related strains, together forming a clonal complex. The low overall rates of horizontal transfer and mutation in *M. bovis* make these complexes easily identifiable, with many strains only varying at a single locus among genetic markers. Strains within the same clonal complex are inferred to have descended from a common founder strain. By identifying hosts (Collins et al., 1988) or geographic regions (Allen et al., 2013) from which members of a clonal complex are reported, we can make epidemiological inferences about the sources of these strains, based on the hosts and locations from which the other members of the complex are reported.

1.2. Possible M. bovis sources

Whether acting as reservoirs maintaining a pathogen or as incidental hosts only occasionally becoming infected, cervids can facilitate *M. bovis* transmission via direct or indirect contact with domestic cattle.

1.2.1. Wild cervids

Outside the US, wildlife reservoirs have been recognized to independently maintain and transmit *M. bovis* to cattle, impeding eradication in cattle populations. These wildlife reservoirs include

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European badger (*Meles meles*) in Europe (Woodroffe et al., 2005), brushtail possum (*Trichosurus vulpecula*) in New Zealand (Collins et al., 1988), Cape buffalo (*Syncerus caffer*) and greater kudu (*Tragelaphus strepsiceros*) in southern Africa (Bengis et al., 1996), elk (*Cervus canadensis*) and American bison (*Bison bison*) in Canada (Wobeser, 2009), and wild boar (*Sus scrofa*) in Europe (Naranjo et al., 2008).

In North America, *M. bovis* has been identified in populations of White-tailed deer (*Odocoileus virginianus*) (Miller and Sweeney, 2013; Smith, 1968), but published data on matching deer bTB genotypes to local cattle are not as extensive as in other bTB wildlife reservoir systems (Biek et al., 2012). Infection in both wild cervids and cattle in these areas have led to trade restrictions and altered wildlife management practices (O'Brien et al., 2006).

1.2.2. Farmed cervids

Farmed cervids are in some aspects managed similarly to cattle, including being fed from shared containers and being transported among farms. Their exposure risks and contact patterns are likely more similar to cattle than to their wild counterparts, so as an epidemiological host group we expect farmed cervids to contribute differently to cattle infection than do wild cervids.

1.2.3. International imports

Cattle imported from *M. bovis*-endemic countries could be periodically introducing the pathogen to US cattle. This would not be a new phenomenon, as pathogen introduction has a long global history as an unintended consequence of live imports. Countries that historically traded with the British Isles, including the US, Canada, New Zealand, Australia, and South Africa, still have *M. bovis* strains in the same clonal complex as strains currently present in the UK (Smith et al., 2011). However, here we are interested in international imports as a source of recent pathogen introductions (within the past two decades), leading to established infections in US cattle populations. Currently, only Canada, Mexico, and Australia are permitted to send live cattle to the United States.

1.3. Evaluating potential external sources

Genetic analyses of *M. bovis* from multiple host species have not previously been evaluated at this large of a spatiotemporal scale for the US. Here we summarize *M. bovis* genetic relatedness, geographic distribution, and host types for the most frequently detected strains in the US, or those causing the highest population-level disease burden. Based on these characteristics, wild cervids, farmed cervids, and imported cattle were all evaluated as possible source populations infecting US cattle.

2. Materials and methods

Data were provided by the National Veterinary Services Laboratories (NVSL) from a collection database of *M. bovis* isolates. These samples are a subset of reported cases in the US between 1989 and 2013. Prior to 2001, isolates were archived at NVSL sporadically with no standardized protocol. Approximately 40% of bTB affected herds between 1989 and 2000 have at least one representative isolate in the database. After 2001 with formalized archiving procedures in place, 100% of US-origin affected herds, and 95% of imported cattle isolates were genotyped and included. The dataset also contained information about individual hosts, including species, production type (i.e., wildlife, game farm), year isolated, last state of residence, and country of origin (Appendix A). Of the 897 *M. bovis* records from cattle, 595 were from US cattle, 202 were from cattle imported from Mexico, 4 were from cattle imported from Canada, and 96 were of unknown origin. We included the strains from Canadian cattle in examining individual strain distributions, but focused on the larger sample from Mexico for further analyses of international imports. We assumed that strains in imported cattle were acquired in the cattle's country of origin, where bTB is endemic (USDA: APHIS, 2013), although the case reports were in the US. All 170 cervid samples, both farmed and wild, were from the US. Eighty-two reports came from other wildlife species (opossums, raccoons, coyotes, and feral pigs); these were not included in the analysis. One hundred ten samples had incomplete genetic data and were excluded from analysis, for a total of 1111 records.

Samples were identified based on spoligotyping, a categorization method commonly used in the *M. tuberculosis* complex (reference database at http://www.mbovis.org (Smith and Upton, 2012)), which includes *M. bovis*. Spoligotypes identify groups of closely related strains based on "presence or absence…of spacer units in the chromosome" (Smith and Upton, 2012). Spoligotypes were grouped into families if they differed from at least one other member by no more than a single spacer deletion (Reyes et al., 2008). This case definition was based on the relative frequency of a single spacer deletion event (ca. 3 times higher) compared to multiple spacer deletion events. Additionally, eleven Variable Number of Tandem Repeat (VNTR) loci, 0424, 0577, 1644 (MIRU16), 1955, 2165 (ETRA), 2401, 2461, 2687 (MIRU24), 2996 (MIRU26), 3192 (MIRU31), 4052 (QUB-26) (Martinez et al., 2008) were characterized and used to further define structure within spoligotype families. Each unique combination of spoligotype and VNTR profile was defined as a strain.

We estimated strain richness among host groups by generating rarefaction curves. This procedure subsamples within a group to estimate rates at which new strains are detected, allowing us to account for different group sample sizes (numbers of reports). We used the "rarecurve" species accumulation curve function in the "vegan" package (Oksanen et al., 2012) in the R programming environment (Oksanen et al., 2012; R Core Team, 2012), to estimate strain detection rates in the host groups: US cattle, cattle imported from Mexico (henceforth "Mexican cattle"), farmed cervids, and wild cervids.

To visualize genetic relatedness among *M. bovis* strains, strains within each spoligotype family were aggregated into clonal complexes using eBURST (http://eburst.mlst.net) (Feil et al., 2004). Relationships among spoligotype families were not evaluated here, but have been described previously (Smith, 2012). Because they were grouped by spoligotype family, strains within the same clonal complex often, but not always, share the same spoligotypes. Relationships within clonal complexes are thus largely defined by VNTR profile similarity, with spoligotype treated as a single locus. Membership within a clonal complex was defined as sharing 11 of 12 loci (11 VNTR plus one spoligotype) with at least one other strain in the complex. We compared strains with respect to host group, production type, and country of origin. We included isolates from cattle of unknown origin to determine the most frequently reported strains, then patterns in those strain distributions were determined based on reports from known locations.

3. Results

3.1. Strain richness

We identified a total of 138 unique strains in 27 spoligotype families. Most spoligotype families were comprised of three to four clonal complexes, and numerous pairs and singleton strains.

The rarefaction curves show large differences in strain richness among US cattle, Mexican cattle, and cervids (Fig. 1). At a sample size of 60 reports from each group, Mexican cattle on average yield