SHORT COMMUNICATIONS

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Brucellosis Transmission between Wildlife and Livestock in the Greater Yellowstone Ecosystem: Inferences from DNA Genotyping

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ABSTRACT: The wildlife of the Greater Yellowstone Ecosystem carries brucellosis, which was first introduced to the area by cattle in the 19th century. Brucellosis transmission between wildlife and livestock has been difficult to study due to challenges in culturing the causative agent, *Brucella abortus*. We examined *B. abortus* transmission between American bison (*Bison bison*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and cattle (*Bos taurus*) using variable number tandem repeat (VNTR) markers on DNA from 98 *B. abortus* isolates recovered from populations in Idaho, Montana, and Wyoming, US. Our analyses reveal interspecies transmission. Two outbreaks (2007, 2008) in Montana cattle had *B. abortus* genotypes similar to isolates from both bison and elk. Nevertheless, similarity in elk and cattle isolates from the 2008 outbreak suggest that elk are the likely source of brucellosis transmission to cattle in Montana and Wyoming. *Brucella abortus* isolates from sampling in Montana appear to be divided in two clusters: one found in local Montana elk, cattle, and bison; and another found mainly in elk and a bison from Wyoming, which is consistent with brucellosis having entered Montana via migration of infected elk from Wyoming. Our findings illustrate complex patterns of brucellosis transmission among elk, bison, and cattle as well as the utility of VNTRs to infer the wildlife species of origin for disease outbreaks in livestock.

Key words: American bison, *Brucella abortus*, cattle, cross-species transmission, elk, infectious disease.

Brucellosis, one of the most-common zoonotic bacterial diseases worldwide (Godfroid 2002), is a major source of concern because of economic losses and health risks. A principal causative agent of brucellosis is the bacterium *Brucella abortus*. Infection has deleterious impacts on reproductive organs and fetal development, leading to reproductive failure (e.g., abortions) in wild and domesticated ungulates (Cheville et al. 1998). Brucellosis in the Greater Yellowstone Ecosystem (GYE) has been a concern following outbreaks in domestic cattle (*Bos taurus*) during the past 25 yr in Wyoming, Montana, and Idaho, US (Cross et al. 2013; Rhyan et al. 2013).

Brucellosis is expanding among Rocky Mountain elk (*Cervus elaphus nelsoni*) in the GYE and re-emerging in GYE cattle after eradication from cattle nationwide briefly in 2008 (Cross et al. 2013). Brucellosis was initially introduced to North American wildlife from cattle in the early 1900s or earlier (Meagher and Meyer 1994). Transmission of *B. abortus* from wildlife to cattle can result in economic loss to producers because it requires slaughtering of infected individuals. Increased disease testing in the GYE helps to protect marketability of cattle (USDA-APHIS 2015).

Until recently, elk were thought to be an insignificant reservoir for *Brucella* transmission to cattle in the GYE (Cheville et al. 1998). However, a recent study suggested that brucellosis outbreaks in cattle in Idaho

(2002) and Wyoming (2003) originated from elk. This was supported by data demonstrating that *B. abortus* DNA profiles from elk isolates were nearly identical (i.e., within one mutational step) to isolates from cattle (Beja-Pereira et al. 2009). Seventeen brucellosis outbreaks in cattle have occurred in the GYE portions of Montana, Idaho, and Wyoming since 2002 (Rhyan et al. 2013). In this study, we genotyped nine variable number tandem repeat sequences (VNTRs), which are used by the National Animal Disease Center for *B. abortus* strain discrimination (e.g., Bricker and Ewalt 2005).

We assessed the wildlife species of origin for these brucellosis outbreaks using a VNTR-9 assay employed by the National Veterinary Services Laboratories (NVSL), Ames, Iowa (Higgins et al. 2012). This new set of loci includes some informative VNTRs used in previous work (Beja-Pereira et al. 2009) and new markers that are variable and provide high resolution for discrimination between strains of *B. abortus* (Whatmore et al. 2006). We analyzed 98 isolates from bison (*Bison bison*), cattle, and elk collected from 1999–2010 across a large area in the GYE (see Supplementary Material Table S1; Fig. S1).

Isolates were collected from hunter-killed elk or during management removals of infected individuals. Isolates from Yellowstone National Park bison were collected from bison captured at the boundary of the park during winter migrations to prevent comingling with cattle in Montana. One bison isolate from the Jackson herd was collected at the National Elk Refuge in 2009 during the hunting season. Cattle isolates were obtained from infected livestock herds.

We obtained VNTR genotypes for 98 isolates from NVSL. A subset of these isolates (n=78) was cultured and identified as *B. abortus* by NVSL while the remaining isolates were processed and identified by Wyoming Game and Fish (n=20). Two biovars were identified in the isolates; biovar 4 was detected in Wyoming cattle and Idaho elk; biovar 1 was detected in Montana (bison, cattle, and elk), Idaho (cattle and elk), and Wyoming (elk). We extracted DNA from each cultured isolate using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) according to the standard manufacturer's protocol. Samples were genotyped using a set of nine VNTRs (see Supplementary Material Table S2) selected for their moderate to high variability and ability to discriminate between strains of *B. abortus* (Bricker and Ewalt 2005; Whatmore et al. 2006).

We analyzed VNTR allelic profiles using Network v4.6 (Bandelt et al. 1999) to construct a haplotype network of genetic relatedness among isolates. We built the network using a median-joining algorithm and used an approach consisting of star-contraction before the median-joining to reduce the overall complexity of the haplotype network (Beja-Pereira et al. 2009). A maximum parsimony post-calculation was also performed to delete all non-maximum parsimony links.

Our network analysis generated a network of 83 haplotypes without preprocessing (see Supplementary Material Fig. S2) and 50 haplotypes using the star-contraction method (Fig. 1). The isolates clustered in two main groups. One cluster was comprised of isolates from the three host species in Montana. The other cluster contained all the isolates from Wyoming and Idaho plus some elk and bison from Montana (Fig. 1).

The clustering of Montana elk isolates (Fig. 1) is consistent with intraspecific transmission among Montana elk (circulating locally) and independent from the Wyoming feed grounds. A recent study in the same area, where there was a distinctness of Montana lineages, suggested that this pattern might be due to low pathogen dispersal (Kamath et al. 2016). These data also supported results from modeling analyses conducted by Cross et al. (2010), suggesting that migration or dispersal of elk from the Wyoming feed grounds alone could not explain the recent increase in *B. abortus* prevalence in Montana elk (Cross et al. 2013). Those results, which showed several Montana elk having *B. abortus* isolates with identical genotypes, indicated a relatively recent transmission event (i.e., a few years). It is possible that homoplasy (i.e., back-mutation or parallel evolution) has caused

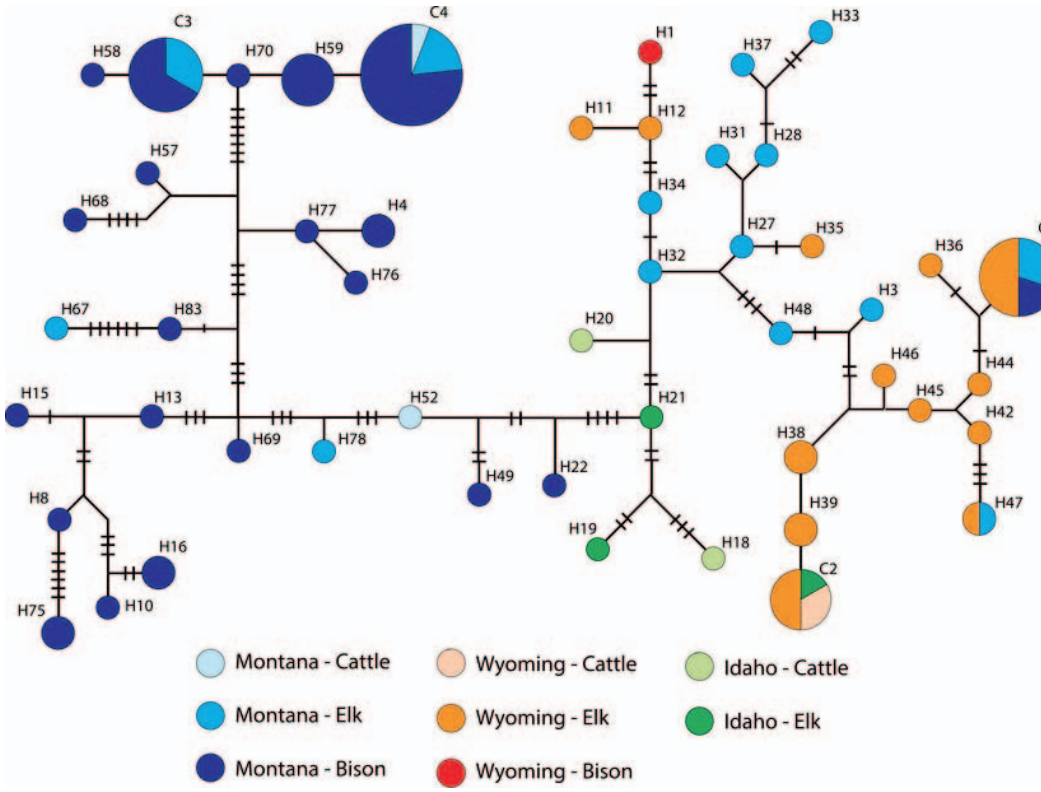

FIGURE 1. Haplotype network of 50 *Brucella abortus* haplogroups from 98 isolates obtained from bison (*Bison bison*), cattle (*Bos taurus*), and elk (*Cervus elaphus nelsoni*) in the Greater Yellowstone Ecosystem using nine informative variable number tandem repeat loci and preprocessing star-contraction (contracted haplogroups are C1, C2, C3, and C4). Each haplotype pie chart (circle) size is proportional to its frequency, and each dash on a branch represents one mutation step.

distinct isolates to have similar VNTR genotypes; however, this is unlikely because multiple VNTR loci were considered and multiple elk pairs each share an identical *Brucella* isolate. Importantly, neither the modeling nor the empirical data we present can differentiate between vertical (mother-to-calf) and horizontal (between unrelated individuals) transmission.

The VNTR profiles of isolates from the Wyoming cattle outbreaks of 2008 group closely with (or are identical to) isolates obtained from elk. Cattle nationwide (including those within the GYE) are strictly monitored. Any *B. abortus* antibody-positive animals are culled; therefore, outbreaks originating from cattle are unlikely. Considerations of this management policy in context with the results from our phylogenetic network analysis suggest that elk were the source of the outbreaks in Wyoming and Idaho. The haplotypes of *B. abortus* were genetically very similar, or identical, to the elk isolate collected in the same area where the cattle isolates were collected. This conclusion is consistent with results of Beja-Pereira et al. (2009), also suggesting that elk were the source of earlier cattle outbreaks in Wyoming and Idaho in 2002 and 2003.

Because *B. abortus* samples from the two Montana cattle outbreaks are genetically similar to isolates from both elk and bison in Montana, these data cannot resolve the wildlife source species of cattle infections in Montana. However, humans have precluded Yellowstone bison from entering any further than a few kilometers into the Paradise Valley of Montana for >100 yr (White et al. 2011).

Also, northern Yellowstone elk do not migrate as far north in the Paradise Valley, where these cattle outbreaks occurred (White et al. 2010).

This finding suggests interspecies transmission between bison, elk, and cattle in the northern portion of GYE and supports the hypothesis that elk in the Paradise Valley are a maintenance host for *B. abortus* and transmit the bacteria to cattle. Results from this haplotype network analysis contradict results presented in Beja-Pereira et al. (2009), which suggested little or no interspecific transmission between elk and bison in the GYE. One plausible explanation for this discrepancy is that sampling in the previous study was limited to a smaller area, and a smaller sample size of isolates, with few samples from the northern region of the GYE. The paucity of historic *B. abortus* isolates from all three species makes it difficult to determine precisely how the increase in infection among GYE elk has occurred, but our study reinforces that elk transmission to cattle is a significant risk for livestock operators to be aware of and to manage. This research underscores the importance of large sample sizes from all three host species and broad geographic coverage in formulating conclusions about *B. abortus* transmission dynamics and potential methods to eliminate brucellosis from the GYE.

In summary, a phylogenetic network analysis based on nine informative VNTR loci for *B. abortus* isolates collected from bison, elk, and cattle in the GYE supports the hypothesis that there is interspecific transmission of *B. abortus* between elk, bison, and cattle. The analysis also suggests intraspecific transmission among elk in populations away from Wyoming feed grounds. Finally, our results suggest elk were the source of brucellosis outbreaks in Idaho, Montana, and Wyoming cattle, which is also supported by a recent study using genomic tools (Kamath et al. 2016).

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2015-12-329.

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