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DNA Genotyping Suggests that Recent Brucellosis Outbreaks in the Greater Yellowstone Area Originated from Elk

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ABSTRACT: Identifying the source of infectious disease outbreaks is difficult, especially for pathogens that infect multiple wildlife species. *Brucella* spp. are among the most problematic zoonotic agents worldwide, and they are notoriously difficult to detect and identify. We genotyped 10 variable number of tandem repeat (VNTR) DNA loci in 56 *Brucella abortus* isolates from bison (*Bos bison*), elk (*Cervus elaphus*), and cattle (*Bos taurus*) to test the wildlife species most likely to be the origin of recent outbreaks of brucellosis in cattle in the Greater Yellowstone Area. Isolates from cattle and elk were nearly identical but highly divergent from bison isolates. These data suggest elk, not bison, are the reservoir species of origin for these cattle infections. This study illustrates the potential power of VNTR genotyping to assess the origin of disease outbreaks, which are increasing worldwide following habitat fragmentation, climate change, and expansion of human and livestock populations.

Key words: Bison, *Brucella abortus*, elk, genotyping, DNA, pathogen transmission, reemerging infectious disease, trace-back study, zoonosis.

Information about the origin and transmission of infectious disease outbreaks is difficult to acquire, especially for diseases like brucellosis that are elusive and infect multiple hosts, including wildlife (Archie et al., 2008). Brucellosis is perhaps the most common zoonotic bacterial disease worldwide, causing widespread human health problems, millions of dollars in losses to livestock industries, and potentially reducing wildlife population reproduction rates (Joly and Messier, 2005; Pappas et al., 2006). Brucellosis infects reproductive organs and causes reproduction failure and abortions in domestic and wild mammals. Brucellosis in the Greater Yellowstone Area (GYA) is caused by *Brucella abortus*, an intracellular, gram-negative bacterium that is difficult to isolate and study because it hides in macrophages and lymph nodes of the immune (reticuloendothelial) system.

Bison (*Bos bison*) and elk (*Cervus elaphus*) are two alternate wildlife hosts capable of shedding and transmitting *B. abortus* in the GYA. Bison often are mistakenly considered to be the likely origin of outbreaks in cattle (*Bos taurus*) because the prevalence of brucellosis is higher in bison (40–60%) than in GYA elk populations (2–30%; Cross et al., 2009). However, bison seldom comingle with cattle because management agencies actively prevent bison dispersal and range expansion outside established conservation areas (e.g., in and near Yellowstone National Park) via hazing, hunting, and/or periodic brucellosis risk-management removals. Conversely, elk often comingle with cattle and can migrate long distances from the 23 winter feeding grounds in northwestern Wyoming where elk are fed hay by state and federal biologists to keep them away from cattle and ranchers' hay stacks. On the elk feed grounds, brucellosis prevalence is relatively high (~20–30%; Thorne et al., 1979; Cross et al., 2009).

The origin (elk versus bison) and management of brucellosis outbreaks in cattle are controversial and uncertain. This is due to a lack of data on *Brucella*

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TABLE 1. Host species, geographic origin, and year of sampling for *Brucella abortus* isolates used in the study.

Host	Geographic origin	Year	No. of *Brucella* isolates
Cattle	Idaho (Freemont)	2002	12
	Wyoming (Muddy Creek)	2003	11
Bison	Montana (Park County)	1992	5
	Montana (Park County)	1995	1
	Montana (Park County)	1997	2
	Montana (Gallatin County)	1999	2
Elk	Idaho (Freemont/Teton Counties)	1999	2
	Idaho (Freemont/Teton Counties)	2000	1
	Idaho (Freemont/Teton Counties)	2001	9
	Idaho (Freemont/Teton Counties)	2002	6
	Wyoming (Sublette County, Muddy Creek)	2003	2
	Wyoming (Lincoln County, Dog Creek)	Unknown	1
	Montana	1992	1
	Montana (Madison County)	1998	3
		Total	56

transmission resulting from the limited sensitivity of molecular diagnostic tools and difficulties in sampling *Brucella* from wildlife species. Here, we present molecular data from highly variable DNA markers that suggest elk are the likely origin of recent outbreaks of brucellosis in Wyoming and Idaho. These data also demonstrate the usefulness of highly variable DNA markers in epidemiologic trace-back studies.

During 1992–2003, we obtained bacteria isolates of *B. abortus* from 25 elk, 10 bison, and 23 cattle from nine locations across the GYA (Table 1). Bison isolates were collected during winter migrations out of Yellowstone National Park, when bison were culled to prevent commingling with cattle (i.e., brucellosis risk-management program). Field strains of *B. abortus* in cattle were isolated during 2 yr of outbreaks, 2002 in Wyoming and 2003 in Idaho. The isolates are from diagnostic specimens that had been cultured, positively identified as *B. abortus*, and archived by the diagnostic laboratory at the National Veterinary Services Laboratories (Animal and Plant Health Inspection Service [APHIS], US Department of Agriculture) in Ames, Iowa.

We genotyped all isolates with 10 variable numbers of repeat loci (VNTR; known as microsatellites in eukaryotes) as described in Bricker and Ewalt (2005). Highly variable VNTRs in *B. abortus* are available thanks to recent genome sequences from *Brucella* species (Halling et al., 2005). The VNTRs are eight-base-pair repeats that are highly variable in number of repeats and thus useful as markers for genotyping (DNA "fingerprinting") and transmission studies of brucellosis. The DNA marker system was called "HOOF-Prints," an acronym for hypervariable octameric oligonucleotide fingerprints. The marker system has remarkably high power of discrimination among isolates and excellent reliability and repeatability (Bricker and Ewalt, 2005).

We analyzed genetic relationships among VNTR allelic combinations (i.e., haplotypes or alleles) using the software NETWORK V4.5 to build a haplotype network. A haplotype network visualizes genetic relationships among distinct isolates (genotypes or haplotypes) using lines to connect haplotypes and cross-hatches on the lines to represent mutational steps (see Fig. 1). The network was constructed using the median-joining algorithm (Bandelt et al., 1999; Almendra et al., 2009), which is considered the most appropriate algorithm to handle multiple-state data

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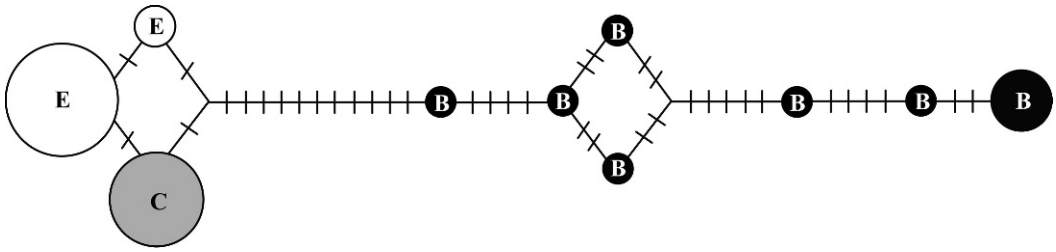


FIGURE 1. Haplotype network for the major *Brucella* haplogroups showing that cattle and elk *Brucella* are nearly identical, but they are highly divergent from all bison *Brucella* isolates. Haplotypes consist of unique multilocus alleles from the 10 VNTR loci. Haplotypes from each host species are shown by a different color and letter: white are elk (E), gray are cattle (C), and black are bison (B). The size of each circle is proportional to the frequency of that haplotype. Each cross-hatch line represents one mutation step, assuming a stepwise mutation model; some loci had more than one mutation step (repeat unit difference) between haplotypes (e.g., between the bison and the cattle/elk haplogroups). Thus, *Brucella* haplotypes of bison all differ by at least 12 mutational steps from elk and cattle *Brucella* haplotypes.

such as ours (e.g., Dos Vultos et al., 2008). The network analyses permit reconstruction of all possible genetic relationships (connecting lines) among haplotypes and also allow the visual representation of the frequencies of each haplotype. Node (circle) sizes indicate the number of bacteria sharing the same haplotype.

Our results indicate that elk and cattle isolates are virtually identical genetically, differing by only one to two mutational steps. On the contrary, bison *B. abortus* differed from cattle and elk by 12–20 mutational steps (Fig. 1). These results suggest that the recent brucellosis outbreaks in cattle in Idaho and Wyoming originated from elk, not bison. *B. abortus* multilocus genotypes from elk remained similar across many years and geographic locations. For example, elk *B. abortus* isolates from Idaho between 1999 and 2002 were almost genetically identical. *B. abortus* isolated in Wyoming elk in 2003 were very similar to *Brucella* from Idaho elk and differed by only one to two mutational steps. These results indicate that the *B. abortus* VNTR loci in elk are reasonably stable between years, and they also suggest that VNTRs are useful for trace-back studies to identify the wildlife species as the source of brucellosis outbreaks around the GYA. The results are also consistent with the fact that elk more often comingle with cattle than do bison because bison management agencies actively prevent dispersal and range expansion outside established conservation areas via hazing, hunting, and/or removals.

The relatively high genetic divergence between elk and bison *B. abortus* isolates suggests that *B. abortus* might not be exchanged extensively between elk and bison, though additional sampling (including more recent bison isolates) and genotyping are required to assess this issue. If true, this finding has important management implications. For example, if transmission between elk and bison is rare, then these two wildlife species might be treated with separate and parallel risk-management and brucellosis-elimination strategies.

Our results illustrate the potential power and promise of molecular genetic markers to assess the origin and spread of infectious disease outbreaks, even for pathogens like *Brucella*, which are difficult to isolate and have genomes with little variation (Archie et al., 2008). In fact, two of 10 VNTR loci were monomorphic among our *Brucella* isolates from GYA bison, elk, and cattle, consistent with the notoriously low polymorphism in *Brucella* genomes. Our study also illustrates that infectious disease outbreaks are increasing worldwide as wild and domestic animals come in closer contact following fragmen-

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tation of wildlife habitats and expansion of human and livestock populations.

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