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Dances with Anthrax: Wolves (*Canis lupus*) Kill Anthrax Bacteremic Plains Bison (*Bison bison bison*) in Southwestern Montana

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ABSTRACT: *Bacillus anthracis,* the cause of anthrax, was recovered from two plains bison (*Bison bison bison*) cows killed by wolves (*Canis lupus*) in Montana, USA, without associated wolf mortality in July 2010. This bison herd experienced an epizootic in summer 2008, killing ~8% of the herd, the first documented in the region in several decades. No wolf deaths were associated with the 2008 event. Surveillance has continued since 2008, with research, ranch, and wildlife personnel diligent during summer. As part of this, we tested wolf-killed bison and elk (*Cervus elaphus*) for anthrax during the 2010 summer using lateral flow immunochromatographic assays (LFIA). Two bison cows were positive for protective antigen, confirming active bacteremia. The LFIA results were confirmed with traditional bacteriology recovering viable *B. anthracis*. No wolf fatalities were associated with the bison deaths, despite consuming the meat. Low-level anthrax occurrence in large, rough terrain landscapes remains difficult to detect, particularly if mortality in the herbivore host is not a consequence of infection. In these instances, surveillance of predators with large home ranges may provide a more sensitive indicator of anthrax emergence or reemergence in such systems. Though speculative, it is also possible that anthrax infection in the bison increased predation risk. These results also suggest *B. anthracis* remains a threat to wildlife and associated livestock in southwestern Montana.

Key words: Anthrax, *Bison bison bison*, *Canis lupus*, plains bison, southwestern Montana, wolf.

While anthrax has been reported from central and eastern Montana, USA, recently (Blackburn et al. 2007), there are few reports from western counties. Limited reports in westernmost counties in the early 20th century (Stein 1945) indicate an early history of the disease regionally. We report the detection of culture-positive bison killed by wolves in southwestern Montana in 2010 on a ranch that had experienced an epizootic in 2008.

Anthrax, caused by the spore-forming bacterium *Bacillus anthracis,* causes mortality in North American wildlife (Hugh-Jones and De Vos 2002) and livestock (Blackburn et al. 2007). Wildlife outbreaks are regular in Texas white-tailed deer (*Odocoileus virginianus*; Blackburn and Goodin 2013) and Canadian wood bison (*Bison bison athabascae*; Dragon et al. 1999). Anthrax is considered an acute disease with potential for large, rapid epizootics. Data from Texas (Blackburn and Goodin 2013) and Ethosha National Park (ENP), Namibia (Turner et al. 2013), suggest that the pathogen circulates annually, with few cases in some years and epizootics in others, likely driven by climate. Serologic surveys have confirmed that several herbivores and carnivores survive sublethal infections, suggesting regular exposure in wildlife (Bagamian et al. 2013).

A major epizootic affected an ~46,539 ha ranch on the Gallatin/Madison county line (45°31′N, 112°18′W) during the 2008 summer, including ~300 plains bison (*Bison bison bison*), 43 or more bull elk (*Cervus elaphus*), two white-tailed deer, and a suspect black bear (*Ursus americanus*). The outbreak began after a midsummer rainy period in a hot, dry summer, classic conditions for anthrax epizootics (Hugh-Jones and Blackburn 2009). Bison deaths were concentrated in two large pastures in the southwest of the ranch, and a third northern pasture erupted late in the outbreak. Elk carcasses were found across the ranch. An estimated 19 wolves were on the ranch in 2008; however, no wolves were documented scavenging on bison carcasses, nor were wolf mortalities attributed to anthrax. An ongoing vaccination program for yearling and adults was implemented in October 2008 with the livestock anthrax vaccine (Colorado Serum Company, Denver, Colorado, USA). Bison calving occurs in spring, and so animals are handled and vaccinated in the fall. Ranch staff also limit access to 2008 outbreak pastures in summer.

Since 2008 we have used routine road surveys and periodic fixed-wing aircraft to search for wildlife mortalities. In 2010 surveillance included testing fresh bison and elk carcasses found during daily bison observations or carcasses found during wolf field studies. Blood and body fluids from fresh wolf kills were screened for protective antigen with LFIAs (Burans et al. 1996). Tissue samples from LFIA-positive carcass remains were sent to the Montana State Veterinary Diagnostic Laboratory, Bozeman, Montana, plated on blood agar, and grown at 37 C for 24–48 hr. Presumptive colonies were Gram stained and evaluated for morphology, biochemical, and string of pearls reactions.

Two of six tested bison carcasses were positive for protective antigen on LFIA kits. An unvaccinated heifer was found on 12 July 2010. A 5-yr-old cow vaccinated in October 2009 was found on 15 July 2010. Viable *B. anthracis* was cultured from both bison. Evidence of struggle at the carcass sites was consistent with wolf kills (McPhee et al. 2012). None of three elk were positive by LFIA or culture for *B. anthracis*. Prior to wolf attack, both bison were grazing and mingling within a large bison group. It is not clear where the bison were exposed to *B. anthracis*. It is plausible that bison were exposed in the days prior to attack when grazing in proximity to the 2008 foci. No wolf mortality was associated with the bison cases.

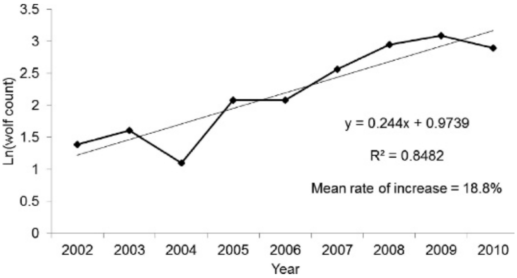

FIGURE 1. Annual growth rate of the Beartrap wolf (*Canis lupus*) pack, Montana, USA, 2002–2010.

At the time of the anthrax-related wolf kills, a conservative estimate of 18 wolves (~12 adults and 6 pups) were present on the ranch. Estimates were derived from visual ground counts conducted throughout the year with subsequent aerial counts performed by Montana Fish, Wildlife and Parks. The pack established itself in 2002, independent of other regional reintroduction efforts. The total number of wolves on the ranch increased from four in 2002 to 18 in 2010, with an estimated mean rate of increase (r) of 0.188 during this period. The number of wolves peaked in 2009, with a decrease to 18 during 2010 ($r=-0.20$), following the methods of Smith et al. (2008; Fig. 1). In 2009 we documented two confirmed wolf-killed newborn bison calves in May and June. Prior to 2009, there was limited wolf/bison monitoring, and we have no definitive records of wolf predation on bison on the ranch. In January 2010 a full-time biologist (V.A.) began monitoring the wolf pack with a focus on predator-prey interactions. In 2010 there were eight confirmed bison calf kills, in addition to the anthrax-positive heifer. There were an additional two bison calves suspected as wolf kills, in addition to the anthrax-positive cow. It is plausible that anthrax infection in these larger bison made them more susceptible to predation by wolves. The possible relationship between bison-disease-predation should be investigated further.

Scavenging anthrax carcasses is a well-documented occurrence in Africa (Bellan et al. 2012), Canada (Dragon et al. 1999), and the US (Mongoh et al. 2008). Birds and carnivores are known anthrax scavengers, with confirmation from serology and culture (see Bagamian et al. 2013). Suspect cases of bear infected from scavenging have been reported in Canada and on this ranch in 2008. Wild dog (*Lycaon pictus*) deaths have been attributed to anthrax in several parks in Sub-Saharan Africa (Creel and Creel 1998). There was no reported scavenging by wolves during the 2008 outbreak, and evidence pointed to live kills in 2010; however, it is plausible that wolves scavenged in 2008. Recently a mathematical model of anthrax in ENP suggested jackal (*Canis* spp.) populations would collapse in the absence of anthrax in herbivores (Getz 2009).

There has been little work done on the short and long-term effects of disease on wolves (Fuller et al. 2003). Other bacterial diseases have been reported, such as brucellosis and tularemia (Zarnke and Ballard 1987), based on serology, though the effects of these diseases are unknown. *Mycobacterium bovis*, the causative agent of bovine tuberculosis, was reported in a wolf pup death (Carbyn 1982). Little is known regarding the effect of anthrax exposure through infected prey on free-ranging wolves, though clinical signs were described for wild (Creel et al. 1995) and domestic dogs (McGee et al. 1994). A single wolf carcass was suspected of anthrax during a plains bison outbreak in Canada in 2008 (Shury et al. 2009). Wolves have been reported scavenging extensively on wood bison carcasses during epizootics in northern Canada with no apparent signs of disease (Dragon et al. 1999). Interestingly, that same study reported that wolves use bison burial mounds associated with outbreak clean-up as lookouts for hunting on the prairie, illustrating a second possible influence of anthrax on wolf predatory behavior.

This report confirms predator kills on bacteremic bison. We reiterate the need for continued surveillance in areas where anthrax may continue at low incidence, particularly in areas where continual vaccination may be required. As illustrated here, there is much to learn about the transmission dynamics of anthrax and the circulation of *B. anthracis* in wildlife. In this study the occurrence of anthrax in this bison population would not have been detected had anthrax surveillance not included sympatric predators and their prey. Predators and prey may provide an important surveillance point for monitoring the occurrence of anthrax in an ecosystem. Lembo et al. (2011) suggested feral dogs may play the same role in Tanzania. Mongoh et al. (2008) confirmed that coyote (*Canis latrans*) presence was significantly increased in pastures with cases during a major epizootic in North Dakota, USA, again identifying a role for canids in surveillance. Low-level anthrax occurrence in large, rough terrain landscapes remains difficult to detect, particularly if mortality in the herbivore host is not a consequence of infection. In these instances, surveillance of predators with large home ranges and tissues from prey may provide a more sensitive indicator of anthrax emergence or reemergence in such systems.

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