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Serologic survey of snowshoe hares in the Greater Yellowstone Area for brucellosis, tularemia, and snowshoe hare virus

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Abstract

We examined sera from snowshoe hares (*Lepus americanus*) live-trapped in the northern Greater Yellowstone Area (GYA) for antibodies to *Brucella abortus*, *Francisella tularensis*, and snowshoe hare virus (SSHV). Zero of 90, 0 of 67, and 40 of 100 samples were positive for *B. abortus*, *F. tularensis*, and SSHV, respectively. Hares were trapped from 2009-2012, and of the 6 animals captured twice with at least a year between captures, 4 developed antibody to SSHV indicating active exposure to the agent. These findings suggest snowshoe hares in the GYA don't play a significant role as a reservoir of *B. abortus*, but do maintain the zoonotic, encephalitic SSHV in the population.

Keywords

Brucella abortus ; brucellosis; *Francisella tularensis* ; Greater Yellowstone Area; snowshoe hare; snowshoe hare virus; tularemia; Yellowstone

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Brucellosis, caused by *Brucella abortus*, is nearly eradicated from livestock in the US. It remains endemic in bison and elk populations in the Greater Yellowstone Area (GYA), which includes Yellowstone and Grand Teton National Parks and portions of Idaho, Wyoming, and Montana near the Parks. Brucellosis was likely first transmitted from cattle to

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wildlife and now occasionally reinfects livestock from wildlife reservoirs. European brown hares (*Lepus europaeus*) and wild boars are known reservoirs for *Brucella suis* biovar 2 in areas of Europe where they pose a risk of infection to livestock (Godfroid, 2005). Infection in hares produces granulomatous lesions in liver, spleen and reproductive organs (Gyuranecz et al., 2011). Serologic surveys in North America have shown rare titers to *Brucella* spp in lagomorphs (Thorpe et al., 1965; Thorne, 2001; Moore and Schnurrenberger, 1981; Aguirre et al., 1992). Thorpe isolated *B. suis* and an unidentified *Brucella* sp. from 2 black tailed jackrabbits (*Lepus californicus*) in Utah (Thorpe et al., 1965). Two serosurveys of snowshoe hares in Alberta, Canada, for *Brucella* antibodies found no positives (Zarnke et al., 1981; Hoff et al., 1970).

Tularemia, caused by *Francisella tularensis*, is another bacterial zoonosis affecting wildlife. Evidence of infection has been found in lagomorphs, rodents, carnivores, ungulates, marsupials, insectivores, birds, amphibians, fish, and invertebrates. Vectors include ticks, biting flies, and possibly mosquitoes. A terrestrial disease cycle involves amplification of the disease in rabbits and hares with arthropods serving as vectors. An aquatic cycle involves the shedding of organisms in the environment by beavers, muskrats, and voles (Petersen and Schriefer, 2005). Two surveys of snowshoe hare populations in Canada have shown zero or low seroprevalence of tularemia (Zarnke et al., 1981; Akerman and Embil., 1982). The organism has been isolated from hares in Alaska and elsewhere (Miller, 1974).

Snowshoe hare virus (SSHV), a mosquito-borne infection in the California serogroup (CAL) of arboviruses (family Bunyaviridae), was first isolated from a sluggish snowshoe hare in Montana in 1959 (Burgdorfer et al., 1961). The infection is usually subclinical in snowshoe hares, and occurs in humans where it is asymptomatic or causes nonfatal encephalitis, usually in children (Meier-Stephenson et al., 2007). Evidence of infection has also been detected in a wide range of wild species (Yuill and Seymour, 2001) and non-fatal clinical encephalitis occurred in two yearling horses in Canada (Lynch et al., 1985; Heath et al., 1989).

This survey was conducted concurrent with a population study that involved annual trapping and ear tagging of hares. Dietary importance of snowshoe hares to mid-sized carnivores, especially lynx (*Lynx canadensis*), in conjunction with the listing of lynx in the contiguous US as a threatened species (Endangered Species Act), has resulted in increased emphasis on hare research and management including population studies (Ruggiero et al. 2000, Zimmer 2004). The study area encompassed 11.7 km^2 (1,172 ha) between Yellowstone National Park and the Absaroka-Beartooth Wilderness in the Bear Creek drainage on the Gallatin National Forest northeast of Gardiner, Montana (Fig. 1). Hares were live-trapped (Tomahawk Live Trap LLC., Hazelhurst, Wisconsin 54531, USA), manually restrained, weighed, measured, sexed, and marked with ear tags. Blood samples were collected from the medial saphenous vein using a 23 gauge needle and 3 ml syringe. Trapping occurred between early January and late March each year from 2009 -2012. Serum was separated and kept frozen (−80C) until shipment to laboratories for testing. Specimens from 2009 to 2012 were tested for antibodies to *B. abortus* by the fluorescence polarization assay (FPA) (Gall et al., 2000) at the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, USA; and for antibodies to SSHV using plaque reduction neutralization test (PRNT) based

on the original procedure described by Lindsey et al. (1976). The neutralizing antibody titers were expressed as the reciprocal of the endpoint serum dilution in 6-well plates that reduced the SSHV plaque count by 90%. Titers ≥10 were considered as positive. Sera from 2009 and 2010 were tested for antibodies to *F. tularensis* by the microagglutination assay. Serologic tests for tularemia and SSHV were conducted at the Centers for Disease Control and Prevention (CDC; Fort Collins, Colorado, USA). During the study, 100-sera were tested for one or more diseases (Table 1). Six hares were sampled twice with captures one or two years apart (Table 2).

All samples tested by FPA (n=90) were negative for *B. abortus* antibodies. All samples tested by the microagglutination assay (n=67) were negative for antibodies to *F. tularensis*. 40% of all samples tested by PRNT (n=100) were positive for antibodies to SSHV (Table 1). Of the six hares sampled twice in different years, one remained negative, one had a similar titer (less than 4-fold change) at recapture, and four converted from negative to positive (Table 2).

Negative findings for *Brucella* antibodies are consistent with two surveys sampling a total of over 1200 snowshoe hares in Alberta, Canada, that found no positives (Zarnke et al., 1981; Hoff et al., 1970). In comparison, European surveys of brown hares in *B. suis* endemic areas found up to 3.5 % seropositive (Gyuranecz et al., 2011; Winkemayer et al., 2005). Another hare species, desert jackrabbits (*Lepus californicus*), experimentally inoculated with *B. abortus*, failed to develop detectable antibodies to the infection nor did bacteria persist in tissues (Thorpe et al., 1967). In the same study, domestic rabbits seroconverted and *B. abortus* persisted in tissues 18 months. Interestingly, *B. suis* biovar 4 infection persisted up to 57 days in experimentally-infected snowshoe hares (Miller and Neiland, 1980). Our results suggest that snowshoe hares play no significant role as wildlife reservoirs for *B. abortus* in the GYA.

Negative findings for *F. tularensis* antibodies are consistent with other serologic surveys of snowshoe hares which found 0 of 11 (Zarnke et al., 1981) and 2 of 1543 (Hoff et al., 1970) hares from Alberta, and 13 of 837 (1.55%) hares from Nova Scotia (Akerman and Embil, 1982) to be positive. Experimental infections of snowshoe hares with *F. tularensis* have demonstrated fatal infections with inoculations of less than 10 organisms. In many cases necropsy revealed no macroscopic lesions (Miller, 1974). The low seroprevalence of tularemia in hares may indicate the species plays little role in the maintenance of the infection in nature or may be due to low survivability following infection (Hoff et al., 1970). Negative serologic surveys in varying hares (*Lepus timidus*) from Sweden have been attributed to the lethal nature of the infection (Mörner et al., 1988).

The finding of seroprevalence to SSHV varying between 19 and 48%, depending on the year, is similar to surveys conducted in Canada and Alaska. In a 9-year study in the 1960's, Hoff and others (1970) found seroprevalence ranged from 20.0 to 75.4% depending on the year, with prevalence decreasing as the hare population increased. Serologic surveys of other snowshoe hare populations have found positive results in 11.3 % (n=1003) in Nova Scotia (Embil et al., 1978), 40% (n=1076) in the Yukon (McLean et al., 1975), 63% (n=11) in Alberta (Zarnke and Yuill, 1981), 65% (n=68) in Alaska (Zarnke et al., 1983), and 55%

(n=20) in Newfoundland (Goff et al., 2012). Seroconversion to positive in four of six hares sampled twice in the current study indicates active exposure to SSHV in the hare population during the years of the study (Table 2).

Investigators in Canada have reported approximately one human case of symptomatic infection with a CAL serogroup virus per year between 1978 and 1989 (Meier-Stephenson et al., 2007). Most of these cases were due to SSHV. Results of the current study should remind health care workers, public health officials, and veterinarians of the presence of an active infection cycle of this mosquito-borne encephalitis virus that is transmissible to and capable of producing clinical encephalitis in humans and horses in the GYA.

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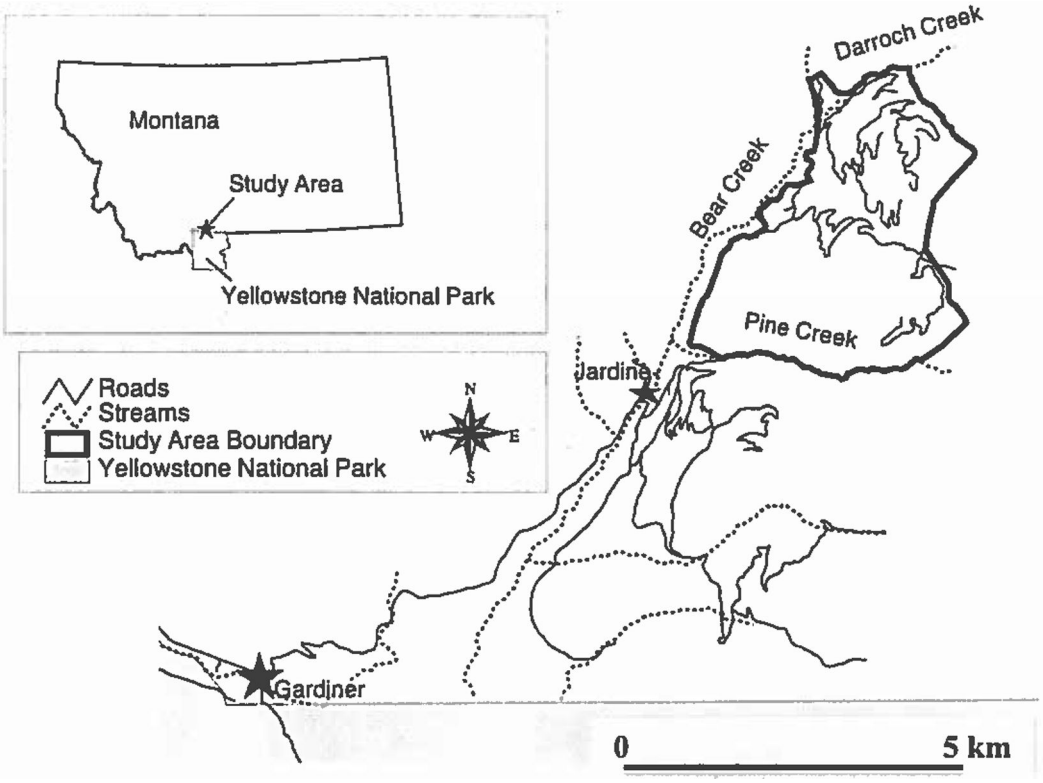

Figure 1.
Map showing location of the Bear Creek study area where snowshoe hares were live-trapped.

Table 1.

Results (number positive/number tested) of serologic survey of snowshoe hares in the Greater Yellowstone Area.[a]

Year	*Brucella abortus*	*Francisella tularensis*	Snowshoe hare virus
2009	0/31	0/36	15/35 (43%)
2010	0/31	0/31	15/31 (48%)
2011	0/21	NE	4/21 (19%)
2012	0/7	NE	6/13 (46%)
Total	0/90	0/67	40/100 (40%)

[a]NE = not evaluated.

Table 2.

Results of serologic tests for SSHV on hares that were recaptured during the study

Animal ID	Date 1st capture	SSHV titer	Date 2nd Capture	SSHV titer
5153	9/22/10	<10	2012	320
5069	3/25/09	<10	3/2/10	80
3711	3/21/09	<10	3/9/10	160
0020	3/24/09	40	3/2/10	80
5168	3/2/10	<10	2/22/11	<10
3475	3/18/09	<10	2/22/11	80