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Jack Rhyan
US Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, Fort Collins, Colorado, jack.c.rhyan@aphis.usda.gov

Dan Tyers
US Forest Service, Interagency Grizzly Bear Study Team, Northern Rockies Science Center, Bozeman, Montana

Jeremy Zimmer
US Forest Service, Custer Gallatin National Forest, Gardiner Ranger District, Gardiner, Montana

Kristen Lewandowski
US Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, Fort Collins, Colorado

Steve Hennager
US Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, Ames, Iowa

See next page for additional authors

Authors
Jack Rhyan, Dan Tyers, Jeremy Zimmer, Kristen Lewandowski, Steve Hennager, John Young, Ryan Pappert, Amanda Panella, and Olga Kosoy

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Jack Rhyan,[1,7] Dan Tyers,[2] Jeremy Zimmer,[3] Kristen Lewandowski,[1,6] Steve Hennager,[4] John Young,[5] Ryan Pappert,[5] Amanda Panella,[5] and Olga Kosoy[5] [1]US Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, Colorado 80521, USA; [2]US Forest Service, Interagency Grizzly Bear Study Team, Northern Rockies Science Center, Bozeman, Montana 59715, USA; [3]US Forest Service, Custer Gallatin National Forest, Gardiner Ranger District, Gardiner, Montana 59030, USA; [4]US Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, 1920 Dayton Avenue, Ames, Iowa 50010, USA; [5]Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, 3156 Rampart Road, Fort Collins, Colorado 80521, USA; [6]Current address: Banfield Pet Hospital, 5270 E Highway 100, Palm Coast, Florida 32164, USA; [7]Corresponding author (email: jack.c.rhyan@aphis.usda.gov)

ABSTRACT: We examined sera from snowshoe hares (*Lepus americanus*) livetrapped in the northern Greater Yellowstone Area (GYA), US, for antibodies to *Brucella abortus*, *Francisella tularensis*, and snowshoe hare virus (SSHV). Zero of 90, 0 of 67, and 40 of 100 samples were antibody positive for *B. abortus*, *F. tularensis*, and SSHV, respectively. Hares were trapped from 2009 to 2012, and of the six animals that were captured twice with at least 1 yr between captures, four developed antibody to SSHV, indicating active exposure to the agent. These findings suggest snowshoe hares in the GYA do not play a significant role as a reservoir of *B. abortus*, but do maintain the zoonotic, encephalitic SSHV in the population.

Key words: *Brucella abortus*, brucellosis, *Francisella tularensis*, Greater Yellowstone Area, snowshoe hare, snowshoe hare virus, tularemia, Yellowstone.

Brucellosis, caused by *Brucella abortus*, is nearly eradicated from livestock in the US; however, *B. abortus* biovars 1 and 4 remain endemic in bison (*Bison bison*) and elk (*Cervus canadensis*) 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 (*Bos taurus*) to wildlife and now occasionally reinfects livestock from wildlife reservoirs. European brown hare (*Lepus europaeus*) and wild boar (*Sus scrofa*) are known reservoirs for *Brucella suis* biovar 2 in areas of Europe where they pose a risk of infection to livestock (Godfroid et al. 2005). Serologic surveys in North America have shown rare titers to *Brucella* spp. in lagomorphs (Thorpe et al. 1965; Thorne 2001). Thorpe and others isolated *B. suis* and an unidentified *Brucella* sp. from two black-tailed jackrabbits (*Lepus californicus*) in Utah (Thorpe et al. 1965). Two serologic surveys of snowshoe hares (*Lepus americanus*) in Alberta, Canada, for *Brucella* antibodies found no positive animals (Hoff et al. 1970; Zarnke and Yuill 1981).

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 mosquitoes. Two serologic surveys of snowshoe hare populations in Canada have shown zero or low prevalence of tularemia, and the organism has been isolated from hares in Alaska and Minnesota in the US and Alberta and British Columbia in Canada (Zarnke and Yuill 1981; Akerman and Embil 1982).

Snowshoe hare virus (SSHV), in the California serogroup 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 it is asymptomatic in humans or causes nonfatal encephalitis, usually in children (Meier-Stephenson et al. 2007). Evidence of infection has also been

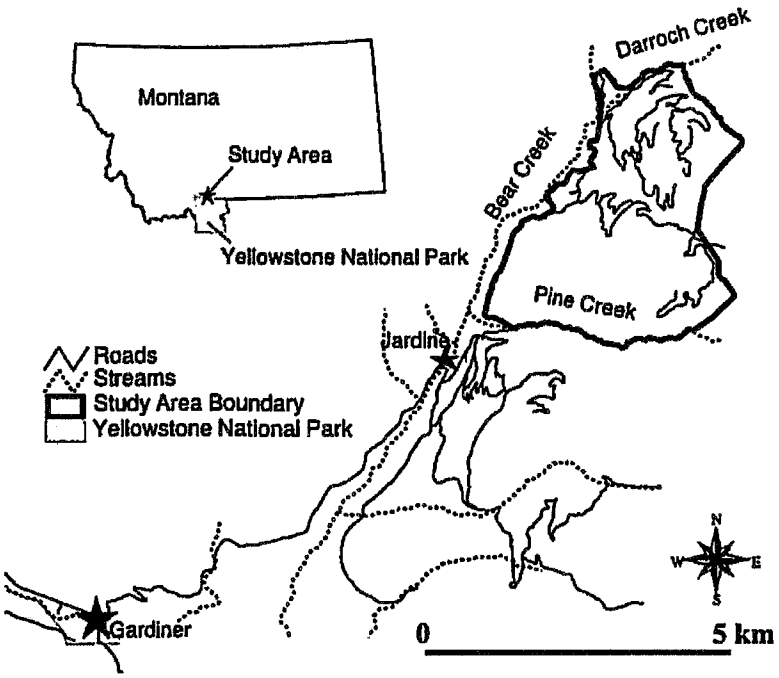

FIGURE 1. Location of the study area in southern Montana, USA, near the northern border of Yellowstone National Park, where snowshoe hares were livetrapped and blood sampled for a serologic survey to detect antibodies to *Brucella abortus*, *Francisella tularensis*, and snowshoe hare virus, 2009–12.

detected in a wide range of wild species (Yuill and Seymour 2001), and nonfatal clinical encephalitis occurred in two yearling horses (*Equus ferus caballus*) in Canada (Lynch et al. 1985; Heath et al. 1989). The virus is carried by several species of mosquitoes in the genera *Culiseta* and *Aedes*, some of which are present in the GYA (Neilsen and Blackmore 1996).

This survey was conducted concurrently with a population study that involved annual trapping and ear tagging of hares. Dietary importance of snowshoe hares to midsized carnivores, especially lynx (*Lynx canadensis*), in conjunction with the listing of lynx in the contiguous US as a threatened species (International Union for Conservation of Nature 2015), has resulted in increased emphasis on hare research and management, including population studies (Ruggiero et al. 2000; Zimmer 2004). The three pathogens were chosen for surveillance due to the endemic nature of *B. abortus* in area wildlife and the public health importance of the agents.

The study area encompassed 11.7 km^2 (1,172 ha) between Yellowstone National Park and the Absaroka-Beartooth Wilderness drainage on the Gallatin National Forest northeast of Gardiner, Montana (Fig. 1; 45°06′N, 110°36′W). Hares were livetrapped (Tomahawk Live Trap LLC, Hazelhurst, Wisconsin, USA), manually restrained, weighed, measured, and marked with ear tags, and then the sex was determined. 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 to 2012. Serum was separated and kept frozen (−80 C) 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) and for antibodies to SSHV by using the plaque reduction neutralization

TABLE 1. Results (number positive/number tested) of a serologic survey for antibodies to *Brucella abortus*, *Francisella tularensis*, and snowshoe hare virus in a population of snowshoe hares sampled in an area in Montana, USA, near the northern boundary of Yellowstone National Park, 2009–12.

Year	*B. abortus*	*F. tularensis*	Snowshoe hare virus (%, 95% CI[a])
2009	0/31	0/36	15/35 (43, 26.6–59.4)
2010	0/31	0/31	15/31 (48, 30.4–65.6)
2011	0/21	NE[b]	4/21 (19, 2.2–35.8)
2012	0/7	NE[b]	6/13 (46, 19.0–73.0)
Total	0/90	0/67	40/100 (40%)

[a] CI = confidence interval.

[b] NE = not evaluated.

test (PRNT) based on the original procedure described by Lindsey et al. (1976). Neutralizing antibody titers were expressed as the reciprocal of the endpoint serum dilution in six-well plates that reduced the SSHV plaque count by 90%. Titers ≥10 were considered positive. Sera from 2009 and 2010 were tested for antibodies to *F. tularensis* by the microagglutination assay. Serologic tests for *B. abortus* were conducted at the National Veterinary Services Laboratories in Ames, Iowa, US, and for *F. tularensis* and SSHV at the Centers for Disease Control and Prevention (Fort Collins, Colorado, USA). During the study, 100 sera were tested for one or more pathogens (Table 1). Six hares were sampled twice with captures 1 or 2 yr apart (Table 2). The time antibody levels would stay elevated in snowshoe hares after exposure to these pathogens is unknown.

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*. Forty percent 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 fourfold change) at recapture, and four converted from negative to positive (Table 2).

Negative findings for *Brucella* antibodies are consistent with two previous surveys that sampled more than 1,200 snowshoe hares in Alberta, Canada (Hoff et al. 1970; Zarnke and Yuill 1981). In comparison, European surveys of brown hares in *B. suis* endemic areas found up to 3.5% antibody positive (Winkelmayer et al. 2005; Gyuranecz et al. 2011). Another hare species, black-tailed jackrabbit (*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). Of note, *B. suis* biovar 4 infection persisted up to 57 d in experimentally infected snowshoe hares (Miller and Neiland 1980). Our results

TABLE 2. Results of serologic tests for antibodies to snowshoe hare virus (SSHV) in hares that were recaptured one or more years apart during a population study on snowshoe hares located in Montana, USA, close to Yellowstone National Park, 2009–12.

Animal	First capture	SSHV titer	Second capture	SSHV titer
5153	22 September 2010	<10	2012	320
5069	25 March 2009	<10	2 March 2010	80
3711	21 March 2009	<10	9 March 2010	160
0020	24 March 2009	40	2 March 2010	80
5168	2 March 2010	<10	22 February 2011	<10
3475	18 March 2009	<10	22 February 2011	80

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 from 0% to 1.55% to be positive (Hoff et al. 1970; Zarnke and Yuill 1981; Akerman and Embil 1982). Experimentally, infections of snowshoe hares with fewer than 10 *F. tularensis* organisms have resulted in fatalities, often with no macroscopic lesions (Miller 1974). The low prevalence of antibody may indicate that snowshoe hares plays little role in the maintenance of *F. tularensis* in nature, or it may be due to low survivability after infection (Hoff et al. 1970), as appears to be the case in mountain hares (*Lepus timidus*) from Sweden (Mörner et al. 1988).

The 19–48% prevalence of antibody to SSHV, depending on the year, is similar to findings in Canada and Alaska. In a 9-yr study, Hoff et al. (1970) found antibody prevalence in the range of 20.0–75.4% depending on year, with prevalence decreasing as the hare population increased. Serologic surveys of other snowshoe hare populations have shown prevalences of 11.3–65% (McLean et al. 1975; Embil et al. 1978; Zarnke and Yuill 1981; Zarnke et al. 1983; Goff et al. 2012). In our study, seroconversion to positive in four of six hares sampled twice indicates active exposure to SSHV in the hare population during the years of the survey (Table 2).

Investigators in Canada reported approximately one human case of symptomatic infection with a California serogroup virus per year between 1978 and 1989 (Meier-Stephenson et al. 2007). Most of these cases were due to SSHV. Our results 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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LITERATURE CITED

Akerman MB, Embil JA. 1982. Antibodies to *Francisella tularensis* in the snowshoe hare (*Lepus americanus struthopus*) populations of Nova Scotia and Prince Edward Island and in the moose (*Alces alces americana* Clinton) population of Nova Scotia. *Can J Microbiol* 28:403–405.

Burgdorfer W, Newhouse VF, Thomas LA. 1961. Isolation of California encephalitis virus from the blood of a snowshoe hare (*Lepus americanus*) in western Montana. *Am J Hyg* 73:344–349.

Embil JA, Embree JE, Artsob H, Spence L, Rozee KR. 1978. Antibodies to snowshoe hare virus of the California group in the snowshoe hare (*Lepus americanus*) population of Nova Scotia. *Am J Trop Med Hyg* 27:843–845.

Gall D, Nielsen K, Fordes L, Davis D, Elzer PH, Olsen SC. 2000. Validation of the fluorescence polarization assay and comparison to other serological assays for the detection of serum antibodies to *Brucella abortus* in bison. *J Wildl Dis* 36:469–476.

Godfroid J, Cloeckaert A, Liautard JP, Kohler S, Fretin D, Walravens K, Garin-Bastuji B, Letesson JJ. 2005. From the discovery of Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Vet Res* 36:313–326.

Goff G, Whitney H, Drebot MA. 2012. Role of host species, geographic separation, and isolation in the seroprevalence of Jamestown Canyon and snowshoe hare viruses in Newfoundland. *Appl Environ Microbiol* 78:6734–6740.

Gyuranecz M, Erdélyi K, Makrai L, Fodor L, Szépe B, Mészáros AR, Dán A, Dencso L, Fassang E, Szeredi L. 2011. Brucellosis of the European brown hare (*Lepus europaeus*). *J Comp Pathol* 145:1–5.

Heath SE, Artsob H, Bell RJ, Harland RJ. 1989. Equine encephalitis caused by snowshoe hare (California serogroup) virus. *Can Vet J* 30:669–671.

Hoff GL, Yuill TM, Iversen JO, Hanson RP. 1970. Selected microbial agents in snowshoe hares and other vertebrates of Alberta. *J Wildl Dis* 6:472–478.

International Union for Conservation of Nature. 2015. *The IUCN red list of threatened species. Version 2014.3.* www.iucnredlist.org. Accessed April 2015.

Lindsey HS, Calisher CH, Mathews JH. 1976. Serum dilution neutralization test for California group virus identification and serology. *J Clin Microbiol* 4:503–510.

Lynch JA, Binnington BD, Artsob H. 1985. California serogroup virus infection in a horse with encephalitis. *J Am Vet Med Assoc* 186:389.

McLean DM, Bergman SKA, Gould AP, Grass PN, Miller MA, Spratt EE. 1975. California encephalitis virus prevalence throughout the Yukon Territory, 1971–1974. *Am J Trop Med Hyg* 24:676–684.

Meier-Stephenson V, Langley JM, Drebot M, Artsob H. 2007. Encephalitis in the summer: A case of snowshoe hare (California serogroup) virus infection in Nova Scotia. *Can Commun Dis Rep* 33:23–26.

Miller LG. 1974. Further studies on tularemia in Alaska: Virulence and biochemical characteristics of indigenous strains. *Can J Microbiol* 20: 1585–1590.

Miller LG, Neiland KA. 1980. Experimental infections by *Brucella suis* type 4 in Alaskan rodents. *J Wildl Dis* 16:457–464.

Mörner T, Sandström G, Mattsson R, Nilsson PO. 1988. Infections with *Francisella tularensis* biovar *palaearctica* in hares (*Lepus timidus, Lepus europaeus*) from Sweden. *J Wildl Dis* 24:422–433.

Nielsen LT, Blackmore MS. 1996. The mosquitoes of Yellowstone National Park (Diptera: Culicidae). *J Am Mosq Control Assoc* 12:695–700.

Ruggiero LF, Aubry KB, Buskirk S, Koehler GM, Krebs CJ, McKelvey KS, Squires JR. 2000. *Ecology and conservation of lynx in the United States.* General Technical Report RMRS-GTR-30WWW. US Department of Agriculture, Forest Service, Rocky Mountain Research Station. Fort Collins, Colorado, 480 pp.

Thorne T. 2001. Brucellosis. In: *Infectious diseases of wild mammals,* Williams ES, Barker I, editors. Iowa State University Press, Ames, Iowa, pp. 372–395.

Thorpe BD, Sidwell RW, Bushman JB, Smart KL, Moyes R. 1965. Brucellosis in wildlife and livestock in west central Utah. *J Am Vet Med Assoc* 146:225.

Thorpe BD, Sidwell RW, Lundgren DL. 1967. Experimental studies with four species of *Brucella* in selected wildlife, laboratory, and domestic animals. *Am J Trop Med Hyg* 16: 665–674.

Winkelmayer R, Vodnansky M, Paulsen P, Gansterer A, Treml F. 2005. Explorative study on the seroprevalence of *Brucella-, Francisella-* and *Leptospira* antibodies in the European hare (*Lepus europaeus* Pallas) of the Austrian-Czech border region. *Wien Tierärztl Mschr* 92:131–135.

Yuill TM, Seymour C. 2001. Arbovirus infections. In: *Infectious diseases of wild mammals,* 3rd Ed., Williams ES, Barker I, editors. Iowa State University Press, Ames, Iowa, pp. 98–118.

Zarnke RL, Calisher CH, Kerschner J. 1983. Serologic evidence of arbovirus infections in humans and wild animals in Alaska. *J Wildl Dis* 19:175–179.

Zarnke R, Yuill TM. 1981. Serologic survey for selected microbial agents in mammals from Alberta, 1976. *J Wildl Dis* 17:453–461.

Zimmer JP. 2004. *Winter habitat use and diet of snowshoe hares in the Gardiner, Montana, area.* MS Thesis, Montana State University, Bozeman, Montana, 65 pp.

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