SHORT COMMUNICATIONS

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Detection of *Rabbit Hemorrhagic Disease Virus 2* in the Pygmy Rabbit (*Brachylagus idahoensis*) in Nevada, USA

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ABSTRACT: *Rabbit hemorrhagic disease virus 2* (RHDV2 or Lagovirus GI.2) began circulating in wild lagomorph populations in the US in March 2020. To date, RHDV2 has been confirmed in several species of cottontail rabbits (*Sylvilagus* spp.) and hares (*Lepus* spp.) throughout the US. In February 2022, RHDV2 was detected in a pygmy rabbit (*Brachylagus idahoensis*). Pygmy rabbits are sagebrush obligates that only occur in the US Intermountain West and are a species of special concern due to the continual degradation and fragmentation of sagebrush-steppe landscapes. The spread of RHDV2 into occupied pygmy rabbit sites may pose a significant threat to their populations because of already declining numbers associated with habitat loss and high mortality rates.

Key words: Great Basin, Lagovirus GI.2, rabbit hemorrhagic disease, sagebrush obligate.

Rabbit hemorrhagic disease virus 2 (RHDV2 or Lagovirus GI.2; Le Pendu et al. 2017; Neimanis et al. 2018), a new variant of classic RHDV (or Lagovirus GI.1), is specific to lagomorphs. This variant was originally detected in northern France in August 2010 in a population of captive rabbits when 25% of RHDV-vaccinated females died (Le Gall-Reculé et al. 2013). It spread throughout France, affecting both captive and wild rabbits, and it almost completely replaced the classic RHDV strain within 1 yr (Le Gall-Reculé et al. 2013). The first confirmed cases in wild lagomorphs in the US were in black-tailed (*Lepus californicus*) and antelope (*Lepus alleni*) jackrabbits and desert cottontails (*Sylvilagus audubonii*) in New Mexico in March 2020. By April 2022, the virus had spread throughout the US, affecting wild populations of hares (*Lepus* spp.) and cottontails (*Sylvilagus* spp.) in New Mexico, Arizona, Texas, Colorado, Nevada, California, Utah, Wyoming, Oregon, Montana, and Idaho (USDA-APHIS 2022). In Nevada, RHDV2 was first detected in June 2020 in a juvenile desert cottontail near the city of Henderson, southern Nevada (N.L. pers. comm.). The first detection in northern Nevada only occurred in March 2021 in a juvenile desert cottontail near Battle Mountain (Fig. 1, site #1; N.L. pers. comm.).

This variant is highly contagious in European rabbits (*Oryctolagus cuniculus*), with an incubation period of 23–48 h (Hall et al. 2021), and it infects rabbits as young as 15–20 d old, which is younger than classic RHDV (6–8 wk old; Lavazza et al. 2021). It withstands harsh conditions and persists in the environment, especially in organic materials, for long periods (Rocchi and Dagleish 2018). This virus is primarily spread through direct contact between lagomorphs, but may also spread through contact with carcasses, environmental reservoirs (e.g., contaminated food, water, feces, soil), and insect or predator feces or be spread by humans (e.g., via tools, boots, vehicles; USDA-APHIS 2020; Taggart et al. 2021). Infected individuals may exhibit weight loss, lethargy, increased body temperature, epistaxis, and seizures (Hall et al. 2021). In European rabbits, mean survival after the onset of clinical signs is 2 d (30–70 h; Hall et al. 2021). Mortality rates among wild and domestic European rabbits and European hare species may reach 80% (Capucci et al. 2017); the mortality rate among native North American lagomorphs is unknown. The introduction of RHDV2 has significant implications for lagomorph species that have restricted ranges and are species of special conservation concern, such as riparian brush rabbits (*Sylvilagus bachmani riparius*; Asin et

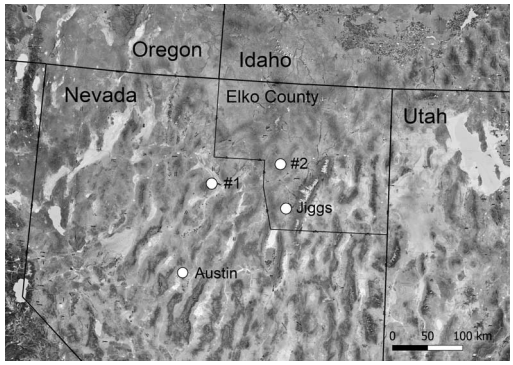

FIGURE 1. The Great Basin, USA, with Elko County, Nevada, and the Jiggs and Austin study sites highlighted. Significant cases of *Rabbit hemorrhagic disease virus 2* in Nevada are also highlighted: first case reported in northern Nevada, in a juvenile desert cottontail (*Sylvilagus audubonii*), near Battle Mountain (#1); first case reported in Elko County in black-tailed jackrabbits (*Lepus californicus*) and the closest report to our study site (#2).

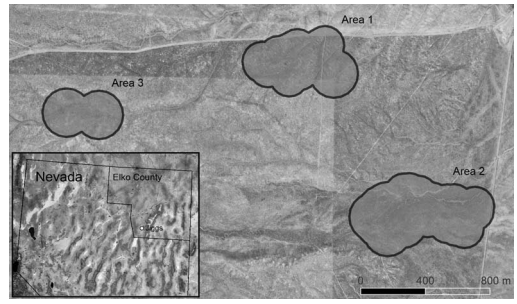

FIGURE 2. Three areas of an occupied pygmy rabbit (*Brachylagus idahoensis*) study site near Jiggs, Nevada, USA. The studied GPS-collared rabbits used these areas pre- and post-*Rabbit hemorrhagic disease virus 2* (RHDV2 or Lagovirus GI.2) detection. These areas were also trapped post-RHDV2.

al. 2021), American pikas (*Ochotona princeps*; Smith 2020), and Columbia Basin pygmy rabbits (*Brachylagus idahoensis*; USFWS 2003).

Pygmy rabbits, the smallest leporid species in North America, exclusively inhabit sagebrush habitats in the US Intermountain West. The species was originally described as *Lepus idahoensis* (Merriam 1891); it was reclassified into the subgenus *Brachylagus* (Miller 1990) and then elevated to a monotypic genus (Lyon 1904). Grinnell et al. (1930) placed *B. idahoensis* in *Sylvilagus*, and the most recent phylogenetic analyses support such placement (Cano-Sánchez et al. 2022). As such, transmission of RHDV2 to pygmy rabbits represents a relatively narrow phylogenetic leap. Pygmy rabbit populations are thought to be declining across their range due to continued habitat loss, degradation, and fragmentation, but they have been denied listing under the Endangered Species Act due to a lack of adequate evidence of decline (USFWS 2010). Pygmy rabbits are nonterritorial and often share burrow systems, putting them in close contact with one another (Sanchez 2007). They are also subject to high rates of mortality (~88%/yr), primarily due to predation (~60%); survivors may live up to 3 yr (Crawford et al. 2010). Introduction of a major new source of mortality such as a virulent virus may lead to widespread extirpation of pygmy rabbit populations.

Monitoring of a population of pygmy rabbits near the town of Jiggs in southern Elko County, Nevada (Fig. 1), starting in 2016, showed a significant decrease in population density from 2016 to 2019 (M.C. pers. comm.). In April–June 2019, we captured two individuals, but in July–December 2021, we captured 17 individuals, with similar trap effort in both years (trap success was 0.05% in 2019 and 1.21% in 2021) across areas 1 and 2 (Fig. 2), indicating that the population may have been increasing from 2019 to 2021. All live-trapping and handling methods were approved by University of Nevada–Reno Institutional Animal Care and Use Committee (00643) and were conducted with proper permits (Nevada Department of Wildlife license 39973).

On 19 January 2022, we radio tracked one of our collared (modified Nano Enhanced GPS collar weight 15 g, Telemetry Solutions, Davis, California, USA) adult female pygmy rabbits and found her dead near an active burrow system in area 2 of the study site (Fig. 2), with no obvious cause of death visible on external examination. Based on the location of consecutive GPS fixes while considering the 2- to 3-m accuracy of the collars, this rabbit probably died 14 January 2022. The rabbit

was frozen and submitted for necropsy to the Nevada Department of Wildlife Health Laboratory, Sparks. On necropsy, we found the carcass was mostly intact other than a 4-cm hole in the abdomen attributed to scavenging. Autolysis was mild to moderate. The lungs contained many multifocal red areas up to 5 mm in diameter evenly distributed throughout all lung lobes, consistent with pulmonary hemorrhage. There was a small amount of hemorrhagic pericardial fluid and a small amount of hemorrhagic abdominal fluid. The liver was a darkened reddish color and was submitted for diagnostic testing, but histopathology was not performed. Real-time reverse transcription PCR (RT-PCR) performed by the California Animal Health and Food Safety Laboratory System, University of California–Davis, found that the rabbit was positive for RHDV2. This detection was reconfirmed by real-time RT-PCR at the National Veterinary Services Laboratories, Foreign Animal Disease and Diagnostic Laboratory, Greenport, New York. The closest previous known detection of RHDV2 had been in black-tailed jackrabbits in Elko County approximately 55 km from our site in June 2021 (Fig. 1, site #2; N.L. pers. comm.).

Two more collared pygmy rabbits were found dead on 19 January and 9 February 2022, respectively, at the entrances of burrow systems in area 2 (Fig. 2), with no evidence of predation. These rabbits were 150 and 155 m from where the RHDV2-positive rabbit died and 25 m apart from one another. For both rabbits, the GPS collars were not collecting locations during this period, so we have no definite evidence that they came into direct contact with the known infected individual. They did use the same areas and were often in close proximity to one another. No viable diagnostic tissues remained on the carcasses, so testing could not be conducted.

After the death of the RHDV2-positive rabbit, on 9–10 February 2022, we trapped from three areas in the study site (Fig. 2) and had 0% trap success. We trapped again on 7 March 2022, but only in area 1 (Fig. 2) because of lack of sign of rabbit activity in the other areas. Two pygmy rabbits were caught; trap success of 4% was highly inflated by targeted trap placement based on pygmy rabbit tracks in the recent snow. Considering the greater number of rabbits captured (n=17) and high levels of activity in all three areas of the Jiggs study site (Fig. 2) before the confirmed RHDV2 infection, compared with the number of rabbits captured (n=2), and only area 1 containing recent pygmy rabbit activity post-RHDV2 detection, it appears that the virus may have had a significant impact on this population.

On 13 January 2022, a day before the death of the RHDV2-positive rabbit, GPS data showed an adult male pygmy rabbit using the same burrow system as the confirmed RHDV2-positive rabbit. The RHDV2-positive rabbit remained near this burrow system for most of 12–13 January, and GPS fixes showed both rabbits at this burrow system less than an hour apart. Despite this evidence of likely contact with the RHDV2-positive rabbit, the male rabbit did not exhibit any external clinical signs when captured on 7 March and survived until 8 April 2022. His collar was found in an open area (350 m from area 1 and 480 m from area 3; Fig. 2) with a few drops of blood on the collar and no carcass remains found in the vicinity, suggesting death by predation. The fact that this rabbit survived for 3 mo after presumably being exposed to RHDV2 suggests that some individuals may survive exposure. The other rabbit captured on 7 March died of unknown causes on 14 March. As of June 2022, we have no collared rabbits at this site and all three previously active areas have no recent sign of pygmy rabbit presence (i.e., fresh fecal pellets or crop marks on sagebrush).

In June 2022, we detected RHDV2 in three dead pygmy rabbits at another study site, near Austin, Nevada (Fig. 1). Necropsy results were consistent with the RHDV2-positive pygmy rabbit at the Jiggs study site. All three animals showed overall darkening of the lungs with multifocal to coalescing dark red discoloration, consistent with pulmonary hemorrhage and congestion. Mild hepatic discoloration was also present. These necropsy findings are consistent with findings in

RHDV2 cases throughout Nevada and elsewhere in North America (see Lankton et al. 2021, fig. 1C). The RT-PCR–positive results combined with consistent gross necropsy findings led us to believe that RHDV2 is the cause of death.

Based on the lack of trap success post-RHDV2 detection, we suspect this disease may be associated with the rapid decline of the Jiggs population. If RHDV2 is associated with high mortality rates in pygmy rabbits, its presence may increase the risk of rapid extirpation across their range. Pygmy rabbit populations should be closely monitored to assess response to this potentially highly contagious and deadly virus. Continued monitoring will also be important to deepen our understanding of how this virus presents in pygmy rabbits, and potentially, infection and mortality rates of this species.

Sagebrush habitats are becoming more fragmented and declining in quality, and some pygmy rabbit populations have been or are becoming isolated from their continuous range. It is imperative that precautions be taken to minimize the spread of RHDV2 within and between populations by humans. Site-specific field gear and clothing should be used when monitoring multiple sites, or all equipment should be disinfected with a 10% bleach solution between sites. When handling rabbits, new disposable gloves should be worn for each rabbit and equipment should be disinfected between handling individual rabbits. All equipment should be soaked in the disinfectant for at least 10 min and then rinsed thoroughly and dried. Field personnel should also avoid contact with domestic rabbits before and after handling wild or feral lagomorphs. In addition, field teams should maintain close communication with wildlife veterinarians for additional precautions to take, handling and testing of potential rabbit carcasses, and whether or how to proceed if RHDV2 is detected in a population. Furthermore, RHDV2 should be considered during land planning and environmental impact assessment because maintenance of connectivity is now more important than ever to allow areas to be repopulated after localized extirpation and to allow for adequate gene flow to improve disease resistance.

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