Case Report

Rabies in Two Bison from Colorado

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Two adult female bison, housed in an outdoor research facility and observed daily, died suddenly three days apart. Minimal coordination and behavioral changes were observed in one animal the evening before being found in a moribund state. Malignant catarrhal fever was suspected in both bison due to a recent confirmed MCF case with similar course. The cause of death was not apparent from necropsy, but brains of both animals were strongly positive for rabies virus antigen by fluorescent antibody and/or immunohistochemical tests. Minimal to mild encephalitis with Negri bodies was observed on histopathology. The bison were located in an area that had not been endemic for skunk rabies; however, a case of rabies in a skunk had been discovered 1.6 km north of the bison paddock two months prior to the bison cases.

1. Introduction

There are few reported cases of rabies in bison (*Bison bison*). A single case occurred in North Dakota in 1998 and was described in a case report [1]. Additionally, 3 cases in South Dakota bison have been reported in surveillance summaries since 1960 [2, 3], and a case in a European bison (*Bison bonasus*) in Russia has been reported [4]. We report here the occurrence of rabies in two bison due to a presumed skunk exposure at an outdoor research facility in Colorado.

2. Case Presentation

The Colorado State University (CSU) Animal Population Health Institute Wildlife Research Facility (APHIWRP) occupies 2.6 hectares and is located on the foothills campus of CSU in Fort Collins, Colorado. At the time of the rabies cases, 60 bison, 12 white-tailed deer, and a colony of 13 feral swine were contained in the facility. The two bison reported

here had been onsite for 9 months. On the morning of June 18, 2012, an adult female bison over 5 years of age was found dead. Disturbance of the soil near the carcass indicated leg paddling prior to death. No abnormalities had been noted in the animals at feeding time the prior evening. At necropsy, the bison had poor body condition, thoracic contusions, and focal fibrinous peritonitis and pleuritis. Rumen contents were considered dry. The cause of death was not apparent based on gross examination. The primary differential diagnosis was malignant catarrhal fever (MCF) due to ovine herpesvirus-2 (OHV-2) as a previous case had occurred in the same herd two months earlier. Tissues were fixed in 10% buffered formalin for histopathology and fresh spleen was submitted to CSU Veterinary Diagnostic Laboratory (CSU-VDL) for OHV-2 PCR.

On the evening of June 20, 2012, a second, adult, female bison from the same paddock was noted to have slight incoordination and uncharacteristic curiosity, approaching an animal caretaker. The following morning the animal

FIGURE 1: Photomicrograph of immunohistochemical stain of cerebellar cortex from the second bison to die from rabies. Many Purkinje cells (large cells) and small nerve cells contain moderate to marked brown staining of rabies antigen in cytoplasm. IHC, antirabies globulin, and Harris hematoxylin counterstain. 200x.

was found moribund and died immediately. On necropsy, there were abrasions and subcutaneous hemorrhage on the lateral and ventral thorax. No other significant lesions were noted and the cause of death was not apparent. Tissues were collected for histopathology and submitted for OHV-2 PCR. Additionally, samples of hippocampus and cerebellum were examined at CSU-VDL for rabies antigen by direct FA staining (DFA) [5].

Spleens from both animals were negative for OHV-2 by PCR. The second bison was positive for rabies by DFA. Formalin-fixed brain tissue from both bison was submitted to the University of Connecticut for rabies IHC. The DFA on bison number 2 and IHC on both bison were strongly positive with unusual abundance of rabies viral antigen in neurons compared to cattle cases (Figure 1). H&E stained sections from both bison had minimal to mild nonsuppurative meningoencephalitis with occasional intraneuronal cytoplasmic Negri bodies.

Sequencing of the viral nucleoprotein and subsequent phylogenetic analysis of the rabies virus by Kansas State University's Rabies Laboratory indicated that the virus was a south central skunk variant [6] similar to another skunk rabies virus from Yuma, Colorado (Figure 2).

Two student externs and one animal caretaker that assisted in the necropsies received postexposure prophylaxis due to the presence of mild abrasions on the arms. All animals in the facility were subsequently vaccinated with a killed rabies vaccine (Imrab Large Animal—Merial Ltd., Athens, GA, USA). Interviews with animal caretakers revealed that a skunk had been observed in the facility on May 25 wandering in a circuitous pattern and unresponsive to human presence.

3. Discussion

Cases of terrestrial rabies have not been reported in the Fort Collins area for approximately 30 years. The first rabid skunk diagnosed in 2012 was found 31 km east of Fort Collins on January 31. Another rabid skunk was found three months later, 48 km west of the original skunk location approximately following the Cache la Poudre river drainage. The second

FIGURE 2: A phylogenetic tree of rabies virus sequences generated from specimens infected with south central skunk variant. A neighbor joining analysis was performed on nucleoprotein gene sequences. Sequences from two raccoon virus variant samples were included as an out group. Samples are identified by their state of origin, animal species, and year of isolation (KSSK11 indicates a skunk from Kansas in 2011). The viral isolate recovered from the second bison in this account is shown in red, while 2 other isolates from Colorado appear in blue. A distance scale is shown at bottom left.

rabid skunk was found 1.6 km from the APhiWRF. According to records at Colorado Department of Public Health & Environment Laboratory and CSU-VDL where testing was performed, 35 rabid skunks were identified between April 25 and November 29, 2012, in Larimer County.

Although no bite wounds were noted on the bison at necropsy, we speculate that a skunk, likely the skunk observed wandering in the facility on May 25, entered the paddock and bit the two female bison that approached to investigate. Investigative behavior of abnormally acting badgers and possums by cattle and red deer resulting in exposure to disease has been described [7, 8]. The short and similar incubation periods in the two bison suggest a common exposure event. Also, the minimal to mild inflammatory changes are consistent with a rapid disease course.

In the previously described case of rabies in a North Dakota bison [1], lethargy, wandering, and remaining apart from the herd was observed for two days before death. Some brain sections had mild to severe disseminated lymphocytic encephalitis. In that case also, the primary differential diagnosis by the veterinarian was MCF.

We think the case reported here of the skunk strain of rabies in two bison is noteworthy due to its short incubation period, rapid time course without clinical signs typically observed in ruminants, abundance of viral antigen demonstrated by DFA and IHC, involvement of more than one animal, and the rapid introduction of the disease to an area not previously endemic.

Conflict of Interests

The authors declare that they have no conflict of interests.

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