Incursion of epizootic hemorrhagic disease into the Okanagan Valley, British Columbia in 1999

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Abstract — In September 1999, unusually high mortality rates in white-tailed deer and California bighorn sheep occurred in the southern Okanagan Valley. Necropsy and histopathologic findings were compatible with epizootic hemorrhagic disease (EHD); the presence of virus was not demonstrated. Subsequent serologic and polymerase chain reaction assays on sentinel cattle suggested an EHD virus incursion.

Résumé — Incursion de la maladie hémorragique épizootique dans la vallée de l'Okanagan en Colombie-Britannique en 1999. En septembre 1999, des taux de mortalité inhabituellement élevés ont affecté le Cerf de Virginie et le mouflon de Californie dans le sud de la vallée de l'Okanagan. La nécropsie et les trouvailles histopathologiques étaient compatibles avec la maladie hémorragique épizootique (MHE); la présence du virus n'a pas été démontrée. Des tests sérologiques subséquents et des analyses par amplification en chaîne par polymérase sur des bovins sentinelles évoquent une incursion du virus de la MHE.

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Epizootic hemorrhagic disease viruses (EHDV) and bluetongue viruses (BTV) are related, *Culicoides*-transmitted, double-stranded RNA orbiviruses belonging to the family *Reoviridae*. Epizootic hemorrhagic disease virus causes an often fatal hemorrhagic disease in white-tailed deer (*Odocoileus virginianus*), an infrequently diagnosed bluetongue-like disease in cattle, and subclinical infections in domestic sheep. Worldwide, 10 serotypes of EHDV are known to exist (1). In North America, serotypes 1 and 2 are enzootic, but only EHDV-2 has been recognized to occur in Canada (2). Epizootic hemorrhagic disease virus causes periodic outbreaks of disease in wildlife, with the last occurrence in Canada taking place in British Columbia (BC) during 1987–1988 (3,4). In addition to its effects on wildlife, EHDV infections of domestic ruminants, particularly cattle, are of concern from a regulatory viewpoint because of serological cross-reactions of EHDV with BTV that may interfere with international movement of animals and their germplasm.

In September 1999, unusual mortalities involving white-tailed deer and California bighorn sheep (*Ovis canadensis californiana*) were reported in the Okanagan Valley of BC. On September 9, 1999, a dead bighorn sheep was noted by a rancher near Okanagan Falls (Figure 1). Five days later, on September 14, the same rancher reported 4 dead white-tailed deer and another dead bighorn sheep. By September 16, an additional 7 white-tailed deer and 1 bighorn ewe were found dead in the same general vicinity as the other dead animals. Lethal levels of nitrates or prussic acid in the sorghum being grown on the ranch were initially suspected as the cause of the deaths. Subsequent analysis of tissue samples revealed that the levels of these substances were within the normal range. On September 18, the BC Ministry of the Environment was informed of another dead bighorn sheep; this animal was necropsied and had lesions consistent with those described for epizootic hemorrhagic disease (EHD). Tissues were collected and sent to the Animal Health Monitoring Laboratory in Abbotsford, BC, and were later forwarded to the National Centre for Foreign Animal Disease (NCFAD) for testing for EHDV and BTV. Additional deaths of white-tailed and mule deer were also reported between September 10 and 20 from Winfield, BC, and the Osoyoos Indian Reserve.

Necropsy findings in the bighorn sheep included: 1) petechial hemorrhages involving the palpebral conjunctiva, salivary glands, tracheal mucosa, testicles, mesentery, subcutaneous tissues, and ruminal and abomasal mucosal surfaces; and 2) ecchymotic hemorrhages involving the apex of the heart, ventricular endocardium, mucosa of the rumen, and the triceps muscle of the right forelimb. Additional postmortem findings included substantial straw-colored fluid within the pericardial sac, hemorrhagic rectal mucosa, and congested to hemorrhagic kidneys. On histopathologic examination of the heart, extensive interstitial myocardial hemorrhages, as well as endocardial, subendocardial, and epicardial hemorrhages, were observed. Sections of the liver revealed focal areas of periacinar hepatocellular necrosis with infiltration of neutrophils, portal vein congestion, and occasional hemorrhages within hepatic

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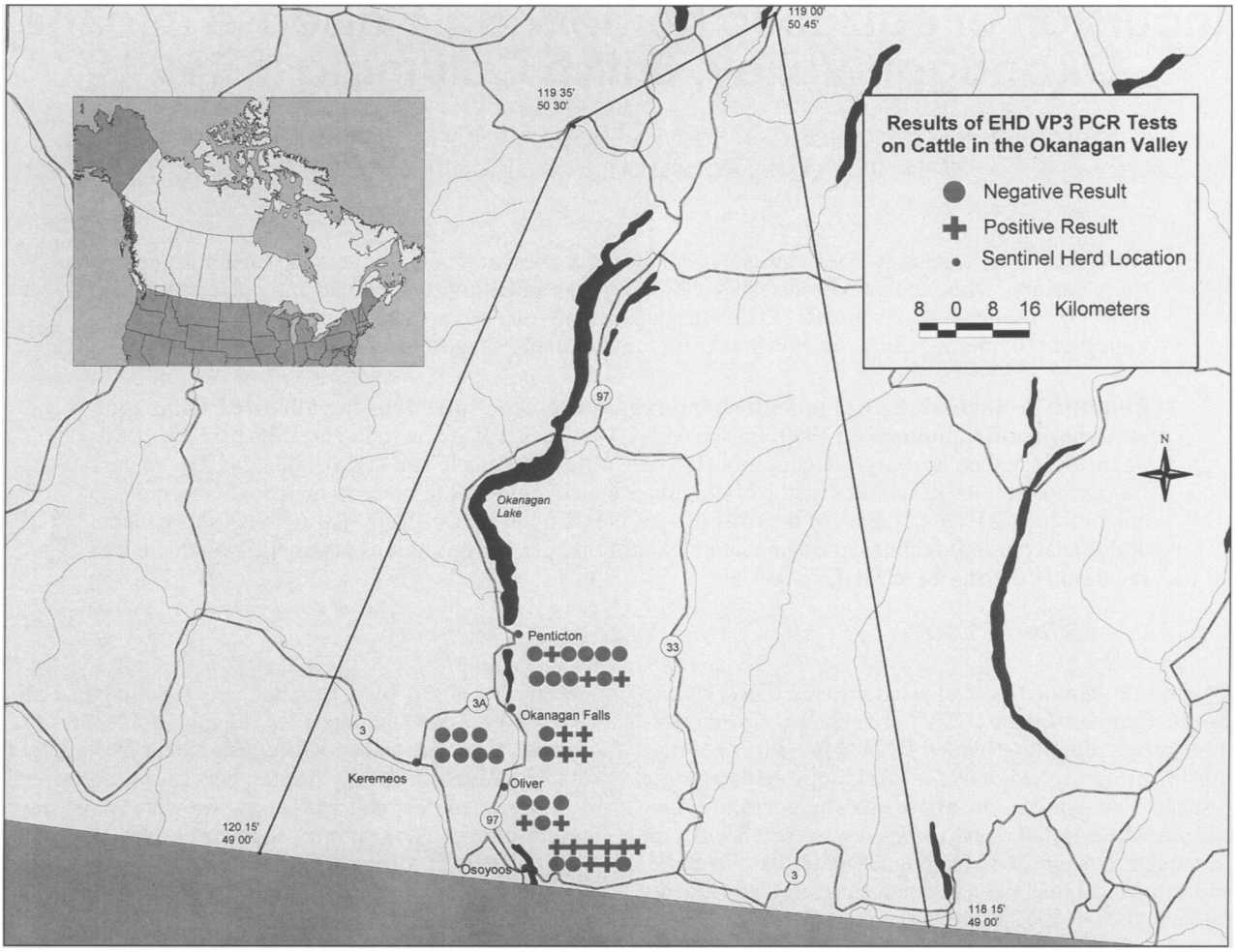

Figure 1. Location of sentinel cattle herds in the Okanagan Valley together with epizootic hemorrhagic disease polymerase chain reaction results for the animals that were tested from these herds.

portal triads. Focal perivascular hemorrhages were also observed in the renal cortex, and paracortical hemorrhages and numerous hemosiderin-laden macrophages were found within the medullary channels of lymph nodes. Similar histopathologic lesions were observed in the tissues from a white-tailed deer. Gross and histopathologic findings in both animals were compatible with those associated with the vascular changes seen with EHD (5); however, attempts to isolate the virus were unsuccessful.

In mid-October, the NCFAD began receiving serum and whole blood samples from sentinel cattle herds situated at several locations along the Okanagan Valley. These samples were processed for antibodies to BTV and EHDV, EHDV isolation, and polymerase chain reaction (PCR) testing. All serum samples tested negative for antibodies to BTV, as determined by a BTV competitive enzyme-linked immunosorbent assay (ELISA) (6,7). By contrast, many of the samples were found to be positive for the presence of antibodies to EHDV, as determined by an EHD competitive ELISA, and an EHDV-2 agar gel immunodiffusion test. Several of these samples were positive for antibodies to EHDV-2, but not to EHDV-1, when tested by a serum neutralization test. Attempts to isolate virus by using washed, sonicated red blood cells inoculated onto BHK-21 cell monolayers were unsuccessful. However, many of the whole blood samples were found to be positive for nucleic acid sequences that were specific for EHDV when an RT-PCR/nested-PCR procedure was used.

The PCR primers used were designed to amplify a stretch of EHDV genomic segment L3 that does not align with the corresponding genomic segment of BTV. The outer set of primers used in the RT-PCR procedure were pm-EHDV-277, which included bases 277 to 295 on the positive-sense strand of genome segment L3 (5′-CATATAGGCATGTGGTGAT-3′), and pm-EHDV-935, which included bases 935–953 on the negative-sense strand of genome segment L3 (5′-CAACGTGATTGA-AGCTATG-3′). The primer set employed in the nested reactions were pm-EHDV-608, which included bases 608 to 626 on the positive-sense strand (5′-CCGT-GATGTCGATGTGTA-3′), and pm-EHDV-851, which included bases 851 to 868 on the negative-sense strand (5′-ATTGAACACCTCGGTACA-3′). The RT-PCR and nested-PCR produced amplicons that were 676 base pairs and 260 base pairs in length, respectively. Both amplicons were cycle-sequenced and the resulting sequences were submitted to the databases of the National Center for Biotechnology Information for sequence comparison using the basic local alignment search tool. The RT-PCR amplicon (GenBank accession number AF258621) was found to be 98% homologous

Table 1. Summary of epizootic hemorrhagic disease serology, and polymerase chain reaction results from sentinel cattle herds in the Okanagan Valley, British Columbia

Animal	Location	EHD-2 AGID	EHD c-ELISA	EHD-2 SN	PCR
285-29	Osoyoos	−	−	−	−
285-30	Osoyoos	S	+	+	+
285-31	Osoyoos	+	+	+	+
285-32	Osoyoos	+	+	+	+
285-33	Osoyoos	+	+	+	+
285-34	Osoyoos	S	+	+	+
285-35	Osoyoos	+	+	+	+
239-15	Osoyoos	−	−	ND	−
239-16	Osoyoos	−	−	ND	−
239-17	Osoyoos	+	+	ND	+
239-18	Osoyoos	−	−	ND	+
237-1	Oliver	ND	+	ND	−
237-3	Oliver	+	+	ND	−
237-4	Oliver	+	+	ND	−
237-5	Oliver	+	+	ND	+
237-6	Oliver	+	+	ND	−
237-7	Oliver	+	+	ND	+
240-23	Okanagan Falls	+	+	ND	+
240-26	Okanagan Falls	+	+	ND	+
286-38	Okanagan Falls	+	+	+	−
286-40	Okanagan Falls	+	−	+	+
286-41	Okanagan Falls	+	+	+	+
286-42	Okanagan Falls	+	+	+	−
238-8	Penticton	−	−	ND	−
238-10	Penticton	S	−	ND	+
238-11	Penticton	+	+	ND	+
238-12	Penticton	+	+	ND	−
238-13	Penticton	+	+	ND	−
281-8	Penticton	+	−	ND	−
281-9	Penticton	−	−	ND	−
281-10	Penticton	+	+	ND	−
281-11	Penticton	S	+	+	+
281-12	Penticton	+	+	ND	−
281-13	Penticton	+	+	ND	−
281-14	Penticton	+	+	ND	−

+ → positive; − → negative; ND → not determined; S → suspicious; EHD-2 AGID → epizootic hemorrhagic disease virus type 2 agar gel immunodiffusion test; EHD c-ELISA → epizootic hemorrhagic disease competitive enzyme-linked immunosorbent assay; EHD-2 SN → epizootic hemorrhagic disease virus type 2 serum neutralization test; PCR → polymerase chain reaction assay

with Alberta EHDV-2 isolate 600544 (GenBank accession number L33820).

Serological, virus isolation, and PCR data for the sentinel herds are summarized in Table 1, and the location of positive PCR reactor animals is depicted in Figure 1.

Although a tentative diagnosis of EHD in deer and bighorn sheep was not confirmed by virus isolation or PCR performed on the tissues from these animals, the results of the tests conducted on serum and whole blood collected from sentinel cattle provide corollary evidence for the incursion of EHDV-2 into the Okanagan Valley in the late summer of 1999. Southern BC appears to have been on the northern edge of an outbreak that was centered in Washington state. Mortalities experienced in the Okanagan Valley as a result of this outbreak are not expected to have significant adverse consequences for local white-tailed deer and bighorn sheep populations.

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