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Mortality in Captive Elk from Salmonellosis

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ABSTRACT: *Salmonella typhimurium* DT104 infections of captive elk (*Cervus elaphus nelsoni*) calves resulted in mortality in eight of 13 affected calves. Salmonellosis in these elk calves was characterized by diarrhea, fever, lethargy, inappetence and depression, and death usually ensued within 72 hr of initial clinical signs. Affected calves did not respond to antibiotic and fluid therapy. The source of the bacteria likely was one or more of the calves when they were captured in the wild at less than 5 days of age. In our captive holding facility, the disease spread quickly and was difficult to control. Phage typing, pulsed field gel electrophoresis, antibiotic sensitivity testing, and plasmid profiles determined that this *Salmonella* sp. strain was the epidemic strain common to cattle, sheep and humans.

Key words: Case history, *Cervus elaphus*, elk, *Salmonella* typhimurium DT104, salmonellosis, wapiti.

Of the 2,435 known serotypes of *Salmonella, S. typhimurium* is one of the most important as a cause of significant economic losses in livestock, and severe clinical disease in humans (Poppe et al., 1998; Murray et al., 1999). *Salmonella typhimurium* phage type 104 (DT104) was initially reported to cause higher hospitalization and mortality among humans than for other forms of salmonellosis (Wall et al., 1994), although more recent evidence failed to support this (Threlfall et al., 1998). This strain has become epidemic throughout North America and Europe (Crerar et al., 1999), and is resistant to at least five antimicrobials, including ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (R-type ACSSuT) (Besser et al., 1997). Based on molecular studies, the resistance genes are chromosomally encoded (Threlfall et al., 1994). In the United States DT104 has been isolated from cattle, sheep, goats, pigs, wild birds, dogs, cats, mice, horses, emu (*Dromaius novaehollandiae*), ground squirrel (*Spermophilus* sp.), raccoon (*Procyon lotor*), and chipmunk (*Tamias* sp.) (Besser et al., 1997). This report describes an outbreak of DT104, which resulted in mortality in eight of 13 captive neonatal elk calves (*Cervus elaphus nelsoni*), also known as wapiti.

In June 1993, 13 elk calves were captured when <5 days of age from a 70 ha enclosure at the United States Forest Service Starkey Experimental Area (Oregon, USA; 45°35′N, 118°10′W). After capture, calves were transported in a covered truck to Washington State University (Pullman, Washington, USA) on 4 June ($n = 3$), 7 June ($n = 3$) and 18 June ($n = 7$). Calves were maintained in a barn with two or three calves per pen and fed fresh goat milk several times per day. Heat lamps were present in all pens. On 10 June, one of the initially captured three calves developed diarrhea at 9 days of age and was treated with electrolyte solution given orally, 5% dextrose solution given subcutaneously, and 2.2 mg/kg of ceftiofur sodium (Naxel, Upjohn, Kalamazoo, Michigan, USA) given intramuscularly twice daily. A fecal sample was submitted to the Washington Animal Disease Diagnostic Laboratory (WADDL) for virus detection by electron microscopy, bacterial isolation, and parasite evaluation. Standard bacterial isolation techniques were used including MacConkeys agar, brilliant green agar and tetrathionate enrichment broth (Murray et al., 1999). A fecal flotation (sugar, sp. gr. = 1.27) was used for fecal parasite evaluation (Foreyt, 1997). During the next 3 wk, all elk calves developed diarrhea and had signs similar to the initial case. Fecal samples were submitted for diagnostic evaluation from all animals within 1 hr of collection. Four of the eight elk that died

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were submitted to WADDL for a complete necropsy evaluation. Selected *Salmonella* sp. isolates ($n = 11$) were submitted for serotyping to the National Veterinary Services Laboratory (Ames, Iowa, USA). The sources of these 11 isolates were the index case on 10 June ($n = 1$), two calves on 14 June ($n = 2$), a calf from the Oregon facility on 16 June ($n = 1$), one isolate from each of the four calves necropsied on 30 June ($n = 4$), one of the five surviving calves on 30 June ($n = 1$), and one elk with transient diarrhea on 22 July ($n = 1$), and 4 August ($n = 1$). The same isolates were phage typed by D. R. Khakhria (Health Canada, Ottawa, Canada), according to the methods described by Khakhria et al. (1997). Plasmid profiles and genomic DNA restriction endonuclease digestion patterns (REDP) by pulsed field gel electrophoresis were determined (Besser et al., 2000).

Serogroup B *Salmonella* sp. isolates were obtained from fecal samples from all 13 elk in this study as well as from one elk from the Oregon facility where the 13 elk were obtained. All of the 11 *Salmonella* sp. isolates subjected to further characterization were determined to be serotype *S. typhimurium,* nine of nine to be phage type DT104, nine of 10 to have the ACSSuT resistance pattern (the exception showing susceptibility to tetracycline), nine of nine to have a single 60 megadalton plasmid, and nine of nine to have REDP characteristics of DT104.

Eight of the 13 elk calves died within the first 30 days of life, usually within 72 hr after developing clinical signs of salmonellosis which included diarrhea, fever, lethargy, inappetence, depression and death. At the beginning of the outbreak, DT104 was isolated from the first calf that developed diarrhea on 10 June when the calf was nine days of age. Viruses were not detected during electron microscopic evaluation and parasites were not detected. Of the five fecal samples submitted from diarrheic elk on 14 June, all were positive for *Salmonella* serogroup B. Elk calf deaths occurred on 21 June ($n = 2$), 22 June ($n = 2$), and 30 June ($n = 4$). Five of the eight calves that died had known birth dates, because they were less than 1 day old when captured, and were 5-, 14-, 16-, 19-, and 22-days-old on the day of death. The remaining three calves were approximately 3-wk-old at death.

At necropsy, all four calves were in fair body condition and weighed between 18 and 25 kg. Lungs of all calves were dark red and had milk or foreign material in the small airways. The small intestines in all calves were dark red, had areas of hyperemia and contained yellow fluid feces. Other organs were considered normal. Histopathological evaluation of tissues revealed severe, diffuse, necrosuppurative enterocolitis with intralesional bacteria and a severe diffuse, pyogranulomatous pneumonia with intralesional bacteria and foreign material in all calves. Mild multifocal hepatic necrosis was observed in the liver of two calves. Intestinal villi were blunted and usually denuded of epithelium. Large areas of severe necrosis throughout the lamina propria and often extending deep into the submucosa were characterized by loss of crypts of Lieberkuhn, fibrin, and pyknotic and karyorrhectic debris. Large colonies of bacteria were present in the mucosa, submucosa and muscularis mucosa of all calves. Multifocal areas of necrosis were also noted in the abomasum of one calf. Parasites were not detected from fecal samples, and DT 104 was isolated from intestinal contents or mesenteric lymph nodes of all four elk. Breda virus detected by electron microscopy from intestinal contents of two calves was considered an incidental finding. The severe necrosuppurative enterocolitis (salmonellosis) was the most significant finding in these elk. However, aspiration pneumonia likely contributed to their deaths.

Of the five surviving calves, DT 104 was isolated from one calf that exhibited transient diarrhea 2 mo after initial isolation, but was not isolated from the other four calves when they were sampled in August

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after they recovered from clinical disease. Viruses were not detected by electron microscopic examination from any of the five surviving calves when they were resampled in August. With the exception of one calf with transient diarrhea, the surviving calves remained clinically normal and healthy.

Important aspects of this outbreak were the rapid onset of disease in the calves, the lack of response to fluid and antibiotic therapy, and the high death rate of affected calves. Although numerous personnel were involved in treating and caring for the calves, the death rate was high and the response to therapy was poor. In general, the calves that died were those most severely affected, despite receiving the most intensive care. Carmalt et al. (2000) indicated that hypernatremia of elk calves was commonly associated with diarrhea, and the protocols used for treating dehydration in bovine calves were unsuitable for elk calves. Bovine calves with diarrhea tend to have normal to low plasma sodium concentrations; whereas, diarrheic elk calves often are hypernatremic. Based on our limited data, we agree with Carmalt et al. (2000). Blood chemistry values were obtained from one of our affected elk calves, and the serum sodium concentration was high (160 mEq/l). Fluid therapy for diarrheic elk calves is imperative, but we recommend that blood chemistry values be evaluated initially and monitored frequently to determine type of fluid therapy, route of administration, volume, composition and concentration of fluids, and duration of treatment. Fluid therapy protocols, other than what we used to treat the elk calves, may have prevented some of the deaths that occurred.

Previous studies have shown that DT104 readily infects humans in contact with infected farm animals (Poppe et al., 1998; Besser et al., 2000). Although DT 104 can cause severe clinical disease in humans, we are not aware of any personnel who developed gastrointestinal disease after the intense exposure to the affected elk and the contaminated facilities.

During the course of the outbreak, additional elk calves that were captured and maintained in the animal holding quarters at the Oregon facility, were also sampled for bacteria and viruses by WADDL by the methods described previously. *Salmonella typhimurium* DT104 was isolated from 1 of 13 fecal samples on 16 June from elk calves approximately 3 wk of age. Because DT104 was also in the source population, it is likely that the bacteria were brought into our facility with one or more of the transported calves. It could not be determined whether the calves acquired the bacteria in the Oregon facility after capture, but before transport to our facilities, or if one or more free-ranging calves was infected before capture. Another possible source of *S. Typhimurium* in our elk calves was the unpasteurized goat milk we fed to them. However, this is an unlikely source because goat kids, lambs, and domestic calves also were fed the same milk composite that was fed to the elk calves on a daily basis, and none of those animals developed diarrhea. Although we have successfully raised elk calves in our facilities in previous years, the stresses of captivity, handling, and human contact may have contributed to the disease.

Salmonella typhimurium DT104 has not been reported from free-ranging elk, and salmonellosis is not currently considered to be a significant mortality factor in captive elk populations (Haigh and Hudson, 1993). Salmonellosis has been documented in three white-tailed deer (*Odocoileus virginianus*) fawns (Debbie, 1968) and one red deer (*Cervus elaphus*) calf (McAllum et al., 1978), but the isolates were not subjected to further characterization. With phage typing and molecular fingerprinting technology, the prevalence of *Salmonella* sp. strains in wildlife can now be more clearly defined. Although phage typing is the definitive method for identifying DT104, our experience has indicated that the ACSSuT resistance and RDEP pat-

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terns are reliably correlated with phage typing. Salmonellosis should be considered in wild ungulates when diarrhea is present, especially in neonatal animals that have a clinical history of enteric disease.

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