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Desert Bighorn Sheep Mortality Due to Presumptive Type C Botulism in California

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ABSTRACT: During a routine telemetry flight of the Mojave Desert (California, USA) in August 1995, mortality signals were detected from two of 12 radio-collared female desert bighorn sheep (*Ovis canadensis*) in the vicinity of Old Dad Peak in San Bernardino County (California). A series of field investigations determined that at least 45 bighorn sheep had died near two artificial water catchments (guzzlers), including 13 bighorn sheep which had presumably drowned in a guzzler tank. Samples from water contaminated by decomposing bighorn sheep carcasses and hemolyzed blood from a fresh bighorn sheep carcass were tested for the presence of pesticides, heavy metals, strychnine, blue-green algae, *Clostridium botulinum* toxin, ethylene glycol, nitrates, nitrites, sodium, and salts. Mouse bioassay and enzyme-linked immunosorbent assay detected type C botulinum toxin in the hemolyzed blood and in fly larvae and pupae. This, coupled with negative results from other analyses, led us to conclude that type C botulinum poisoning was most likely responsible for the mortality of bighorn sheep outside the guzzler tank.

Key words: Bighorn sheep, botulinum toxin, *Clostridium botulinum* type C, mortality event, *Ovis canadensis.*

The Old Dad Peak bighorn sheep (*Ovis canadensis*) population occupies a mountainous area 30 km south of Baker in eastern San Bernardino County (California, USA; 35°06′N, 115°45′W) (Bleich et al., 1997). It has been one of the largest and most productive desert bighorn populations in California, recently estimated at 201 to 300 animals (Torres et al., 1994), and has been the source of translocation stock for reintroduction efforts in mountain ranges from which bighorn sheep have been extirpated (Bleich et al., 1990). More than 200 individuals have been translocated from this population since 1983 (Torres et al., 1996).

Four artificial water catchments, commonly referred to as guzzlers, have been installed at or near Old Dad Peak, because natural water sources are few (Weaver et al., 1969). Guzzlers capture runoff from rainstorms and store the water in covered, heavy duty plastic tanks that are connected to a float-valve controlled basin (Bleich and Pauli, 1990) from which wildlife drink. Bighorn sheep use these guzzlers extensively, especially during hot periods of the year (Bleich et al., 1997). Guzzlers found in the vicinity of Old Dad Peak include the Old Dad Peak guzzler, the Kelso Peak guzzler (13 km east), the Vermin Tank guzzler (4 km north), and the Chuck Kerr guzzler (7 km southeast).

This population of bighorn sheep has been the focus of ongoing, long-term ecological research efforts (Bleich et al., 1997). To facilitate these studies, radiocollars (Telonics, Inc., Mesa, Arizona, USA) have been maintained on a cohort of bighorn sheep and are monitored by fixed-wing aircraft on a regular schedule. Monitoring during the months of June and July were cancelled due to vacations and aircraft maintenance. During a routine telemetry flight on 25 August 1995, mortality signals (higher transmitter pulse rate triggered by 6 hr without motion) were detected from two of 12 radio-collared females. Field investigations ensued between 29 August and 11 December 1995 to document the mortalities and to collect samples for diagnostic evaluation. Ultimately, one raven (*Corvus corax*) and 42 bighorn sheep were found dead in the vicinity of the Old Dad Peak guzzler and three bighorn sheep were found dead near the Vermin Tank guzzler. No mortalities were found near the Chuck Kerr or Kelso guzzlers. These mortalities consisted of both sexes (n = 45; 16 females, 10 males, 19 undetermined) and all ages (<1-to >15-yr-old; age estimated by horn annuli method of Geist, 1966). The carcasses found outside the guzzler tank were desiccated and intact, suggesting that the mortality occurred several weeks prior to their discovery on 29 August 1995. Upon examination of carcasses, no evidence of scavenging or predator-related mortality was detected.

The Old Dad Peak guzzler is located on a remote, steep limestone mountain mass. It includes three above-ground plastic storage tanks (6,500 L). Two of the three storage tanks had insufficient water to feed the drinker basin. The third tank was full, but the valve connecting it to the system was closed, leaving the drinker basin dry. The hatch cover on top of this tank was dislodged, allowing access to the interior of the tank. The top portion of this tank also was indented and supported a pool of algae-covered water containing hundreds of dead fly larvae. Putrified remains of 13 lambs were found in this tank.

Our list of differential diagnoses for the bighorn sheep that died around the two guzzlers included acute infectious disease (e.g., *Pasteurella* spp. pneumonia), acute toxicosis (including blue-green algae poisoning and botulism), gunshot, lightning strike, water deprivation (dehydration), and deliberate poisoning. Examination of carcasses revealed no evidence of gunshot wounds or lightning strike. Death due to water deprivation was considered unlikely because of the presence of alternative water sources in the region (other guzzlers and barrel cacti [*Ferocactus cylindraceus*]), proximity of some of the carcasses to the Vermin Tank guzzler that contained fresh water, and evidence (Krausman et al., 1985) that desert bighorn sheep can, at least in some situations, persist without permanent surface water.

Samples of water, carrion, fly larvae, and pupae were collected between 31 August and 14 September from the contaminated tank of the Old Dad Peak guzzler. The desiccated carcasses did not yield useful samples. However, on 5 September 1995, a recently dead (several hours) bighorn female was found near the Old Dad Peak guzzler following availability of putrid water in the drinker basin. Access to this putrid water was made possible when the contaminated tank was drained during one of the earlier field investigations. This carcass was flown by helicopter to the California Veterinary Diagnostic Laboratory (CVDLS; San Bernardino, California, USA) for complete necropsy. The contaminated tank was dismantled and cleaned on 23 September 1998, and the contents were evaluated. We used tooth development patterns (Deming, 1952) to determine ages of sheep removed from the tank.

Using gas chromatography (Model 5890 MSD, Hewlett-Packard, Palo Alto, California, USA), no detectable levels of organophosphates, carbamates, toxaphene, chlordane, lindane and strychnine were found in water from the contaminated tank. There were no detectable levels of ethylene glycol (gas chromatograph/mass spectrometer; Model 5890 MSD, Hewlett-Packard), mercury (inductive coupled plasma emission spectrometry; ICP-AES; Accuris, ARL/Fisons, Detroit, Michigan, USA) and nitrates/nitrites (ion chromatography; Dionex 4000 Series Ion Chromatograph, Sunnyvale, California, USA). The water samples contained acceptable levels of sodium and salts (ICP-AES), with the exception of elevated levels of potassium and phosphorus, most likely a result of decomposition of the carcasses. Blue-green algae toxicity (Carmichael, 1994) was ruled out based on negative plant identification and negative results from mouse bioassay tests (Cook et al., 1989).

Water samples collected from the Old Dad Peak guzzler were tested for type C botulinum toxin by mouse bioassay (Quortrup and Sudheimer, 1943) and by ELISA (Rocke et al., 1998) at the National Wildlife Health Center (NWHC; Madison, Wisconsin, USA). California Department of Fish and Game (CDFG; Rancho Cordova, California, USA) tested numerous times for type C botulinum toxin using mouse bioassay (Quortrup and Sudheimer, 1943). CVDLS (Davis, California, USA) tested the water sample once by mouse bioassay (Hatheway, 1979). All filtered (0.45 μm filter) and unfiltered water subsamples tested were negative by both mouse bioassay and ELISA.

Seven fly larvae and/or pupae samples were ground, diluted with saline (1:2–1:10 w/v), centrifuged, and for most samples, the supernatant was filtered through a 0.2 μm or 0.45 μm millipore filter. One fly larvae and one pupae sample tested positive for type C botulinum toxin by mouse bioassay (CDFG) using monovalent antiserum by the method of Quortrup and Sudheimer (1943), whereas all other fly larvae samples tested by mouse bioassay were negative (CVDLS, CDFG, and NWHC). A fly larvae sample tested by ELISA was negative (NWHC).

There were no significant findings on gross necropsy and histopathologic examination of the freshly dead bighorn female, except for a small (2 cm) solitary caseous abscess present in the left mandibular muscle, which on culture yielded moderate growth of *Actinomyces pyogenes* using standard bacteriologic methods. The carcass was in good nutritional condition with full forestomachs containing relatively dry roughage. The abomasum contained a small amount of moist, greenish ingesta. A mixed bacterial flora was isolated from the liver and lungs using standard bacteriologic methods. The intestinal pool was negative for *Salmonella* spp.

Hemolyzed blood from the carcass was tested for type C botulinum toxin by mouse bioassay at NWHC, CVDLS, and National Marine Fisheries Service (NMFS; Seattle, Washington, USA) and by ELISA at NWHC as described previously. Initial testing by mouse bioassay was inconclusive because both antitoxin-protected and unprotected mice died from non-specific infections. However, with dilution and antibiotic treatment, the hemolyzed blood tested positive for type C botulinum toxin at NWHC and NMFS. In addition, the hemolyzed blood sample was strongly positive for type C botulinum toxin by ELISA (NWHC). Based on the presence of type C botulinum toxin in the hemolyzed blood of one dead bighorn female and in two fly larvae/pupae samples from the Old Dad Peak guzzler, the lack of any other plausible cause of death, and other epidemiologic observations, including the dead lambs in the guzzler, we conclude that type C botulinum toxin was most likely responsible for the death of bighorn sheep found outside of the contaminated tank.

Botulism is caused by a neurotoxin produced by the bacterium, *Clostridium botulinum.* This neurotoxin interferes with the release of acetylcholine at the neuromuscular junction, resulting in muscle paralysis (Simpson, 1981). There are seven serologic types of botulinum toxin identified, referred to as types A, B, C (C1 and C2), D, E, F, and G. The geographic distribution of these types of toxins apparently varies. In the southern hemisphere type C and D toxin are most often diagnosed in botulism epizootics in livestock. In the United States and Europe, type B toxin is most often diagnosed (Kriek and Odendaal, 1994). In California, type C toxin is most commonly involved in botulism epizootics of free-flying waterfowl, in which the source of the toxin is often maggot-infested carcasses (Duncan and Jensen, 1976).

Clostridium botulinum is an obligate anaerobe that forms resistant spores in adverse environmental conditions. These spores are widely distributed in the environment and are commonly carried in tissues and intestines of animals (Kriek and Odendall, 1994). Animal carcasses have been shown to be a suitable substrate for the production of type C botulinum toxin and have been implicated in type C epizootics in livestock (Kriek and Odendall, 1994) and other animals. In addition, fly larvae that feed on decaying carcasses can ingest and accumulate toxin in the process and have been shown to be a significant source of type C toxin in botulism outbreaks in waterfowl (Reed and Rocke, 1992). Wobeser et al. (1997) described a type C botulism epizootic in cattle that occurred in association with botulism in free-flying waterfowl. The source of the type C toxin was suspected to be duck carcasses or toxin-laden maggots.

Botulism has not been previously reported in bighorn sheep, but mortality events among domestic sheep have been reported. Botulism in domestic sheep was first documented in Australia in 1928 and was later responsible for large epizootics until vaccination was instituted (Bennetts and Hall, 1938). These deaths resulted from the ingestion of type C botulinum-contaminated carrion, generally rabbit; however, on several occasions carrion-contaminated water was implicated. In South Africa, botulism has been reported to cause sporadic, but heavy, losses in domestic sheep (Van der Lugt et al., 1995). This bighorn sheep mortality event was not discovered until several weeks after its occurrence, making reconstruction of the episode difficult and speculative. The selection of suitable samples for diagnostic evaluation was limited; only one carcass was suitable for necropsy. Intense rainstorms occurred in mid-August 1995, which may have drastically altered environmental conditions that existed prior to the field investigations. In addition, the mortality event was of a mixed etiology, with 13 lambs dying from presumptive drowning within the guzzler tank. Events that could have caused this mortality are presented in the following reconstruction.

Initially the drinker basin went dry, and water was no longer available to the bighorn sheep at the Old Dad Peak guzzler. Perhaps sensing water in the one full tank, sheep jumped onto the top of the tank from a nearby rock ledge, eventually denting the center portion. At some point the hatch cover was dislodged, thereby making the water available through a circular opening approximately 50 cm in diameter. As the water level receded, lambs had greater difficulty reaching the water through the opening, fell into the tank while attempting to drink, and drowned. The decaying lamb carcasses served as the substrate for the growth of *C. botulinum* and for the production of type C botulinum toxin. Clark (1987) demonstrated that botulinum toxin is produced in 2 days or less at 37 C. The normal daytime ambient temperature for the Old Dad Peak area often exceeds 38 C in the summer (Weaver et al., 1969). The adult sheep were subsequently exposed to botulinum toxin either by inadvertently ingesting toxic carrion or toxin-laden fly larvae, while attempting to drink from the contaminated tank.

The variable incubation period (12 hr to several days) reported for botulinum toxin (Smith, 1977) could account for the dispersion of sheep carcasses within the Old Dad Peak guzzler canyon and three carcasses at the Vermin Tank guzzler site located 4 km to the north. The intense August rainstorms reported would account for the presence of water containing fly larvae in the dented portion of the tank. As the tank collected water during the rainstorms, the water level inside rose and spilled through the open hatch onto the then concave surface of the tank due to the obstruction of the overflow pipe by floating debris from dead lambs.

Although conclusive evidence is lacking and our diagnosis is presumptive, we believe this mortality in bighorn sheep is consistent with botulism intoxication. Decomposing carcasses suitable for botulinum toxin production were present in the water source proximate to most of the bighorn sheep carcasses. Type C botulinum toxin was detected in fly larvae and pupae collected at this water source and also in the hemolyzed blood of the one bighorn sheep suitable for testing. No significant pathologic lesions were evident in this animal upon necropsy and other plausible causes were ruled out during field investigations and by diagnostic testing. Although we can not rule out the possibility that the other bighorn sheep died from some other cause, the condition and dispersion of carcasses indicated a rapid onset of mortality, typical of an intoxication.

The occurrence of this mortality event demonstrates the importance of guzzler placement during installation. Access to the tank tops by bighorn sheep can be prevented by placement of the guzzler tanks away from ledges or rocks. In areas where the canyons are narrow, a platform bolted to the tank top can be installed to prevent bighorns from breaking through the top of the tank. In addition, the hatch covers can be fastened securely with safety locks. An event such as this was unforeseeable, but should now be a consideration for existing guzzlers and for future installations.

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