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Septicemic Pasteurellosis in Free-ranging Neonatal Pronghorn in Oregon

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ABSTRACT: As part of a study to determine the cause(s) of population decline and low survival of pronghorn (*Antilocapra americana*) neonates on Hart Mountain National Antelope Refuge (HMNAR), Oregon (USA), 55 of 104 neonates captured during May 1996 and 1997 were necropsied ($n = 28$, 1996; $n = 27$, 1997) to determine cause of death. Necropsies were conducted on fawns that died during May, June, or July of each year. The objectives of this study were to report the occurrence and pathology of pasteurellosis in neonates and determine if the isolated strain of *Pasteurella multocida* was unique. Septicemic pasteurellosis, caused by *P. multocida,* was diagnosed as the cause of death for two neonates in May and June 1997. Necropsy findings included widely scattered petechial and ecchymotic hemorrhages found over a large portion of the subcutaneous tissue, meninges of the brain, epicardium, skeletal muscle, and serosal surface of the thorasic and abdominal cavities. Histological examination of lung tissues revealed diffuse congestion and edema and moderate to marked multifocal infiltrate of macrophages, neutrophils, and numerous bacteria within many terminal bronchioles and alveoli. *Pasteurella multocida* serotypes A:3,4, and B:1 were isolated from several tissues including lung, intestinal, thorasic fluid, and heart blood. Each B:1 isolate had DNA restriction endonuclease fingerprint profiles distinct from isolates previously characterized from domestic cattle, swan (*Olor* spp.), moose (*Alces alces*), and pronghorn from Montana (USA). This is the first report of pasteurellosis in pronghorn from Oregon and the B:1 isolates appear to be unique in comparison to DNA fingerprint profiles from selected domestic and wild species.

Key words: Antilocapra americana, Pasteurella multocida, pasteurellosis, pronghorn, survey.

Recent declining numbers of pronghorn (*Antilocapra americana*) and low survival of neonates (<1:100 does in 1995) on Hart Mountain National Antelope Refuge (HNMAR) in south-central Oregon (USA; 42°30′N, 119°40′W) prompted a study to determine the cause(s) of population decline and low survival of neonates. The objectives of this study were to report the occurrence and pathology of pasteurellosis in neonates and determine if the strain of *Pasteurella multocida* isolated from neonates was unique to pronghorns.

Pasteurellosis refers to pneumonia, septicemia, and other infections caused by bacteria of the genus *Pasteurella. Pasteurella* spp., especially *P. multocida* and *P. haemolytica,* have been incriminated as important pathogens of both domestic and wild animals in North America. Large animals affected include domestic cattle and sheep (Yates, 1982; Ellis, 1984), Rocky Mountain bighorn sheep (*Ovis canadensis*) (Post, 1962; Spraker et al., 1984; Dunbar et al., 1990), Rocky Mountain elk (*Cervus elaphus*) (Post, 1960; Franson and Smith, 1988; Wilson et al., 1995), American bison (*Bison bison*) (Heddleston and Gallagher, 1969), and pronghorn (Powell, 1954; Thorne, 1982).

One hundred-four neonatal pronghorns (<1- to 4-days-old) were captured during May 1996 and 1997 by search crews with hand held nets. Biological measurements were recorded, and blood was collected from the jugular vein of each neonate. Also, a radio transmitter (Advanced Telemetry Systems, Isanti, Minnesota, USA) was attached to the ear of each neonate. Neonates with attached radio transmitters were located, but not disturbed, twice daily, until death, or until mid-July each year when radio transmitters lost power. Each animal was recovered as soon as possible after a radio-signal indicated probable mortality.

Necropsies were performed on 55 neonates, 28 in 1996 (12 male, 15 female, 1 undetermined sex) and 27 in 1997 (13 M, 12 F, 2 undetermined sex). Some whole carcasses ($n = 13$) and partially intact carcasses ($n = 42$), consisting mostly of head and neck, were recovered. Otherwise, due to scavenging by animals, only scattered remains, including bones and teeth, were found from the remaining 32 dead neonates. These limited remains often made a diagnosis of cause of death impossible. If enough of the carcass was intact, a necropsy was either performed immediately ($n = 15$) or the carcass was frozen at −20 C and necropsy performed later ($n = 40$).

During necropsy, rayon tipped swabs in Amies transport medium (Cultureswab, Difco®, West Molesey, Surrey, UK) were routinely taken of tonsillar crypts ($n = 29$) and aqueous humor ($n = 11$), for routine microbiological culture because, in most neonates, the head was all that remained to determine a diagnosis. The tonsils were cultured in an attempt to isolate *Pasteurella* spp. to determine which species, biotype, and serotype, may be isolated from an individual diagnosed with or without pasteurellosis. The aqueous humor was cultured because when a head and neck only were recovered, this was the only tissue that could be assumed to be aseptic or nearly so; Therefore, *Pasteurella* spp. that may be isolated from the aqueous humor may indicate septicemia. Thus, a presumptive, but not definitive diagnosis could be made.

A definitive diagnosis of septicemic pasteurellosis was made upon observing typical lesions as described by Thorne (1982), and isolation of *Pasteurella* spp. from tissues other than tonsils or naso-oropharyngeal area where isolation can be found even in apparently healthy animals (Onderka and Wishart, 1988; Dunbar et al., 1990). *Pasteurella* spp. have been isolated from apparently healthy adult pronghorns in Oregon (U.S. Fish and Wildlife Service, unpubl. data). Cause of death due to predation, and which predator was involved, was determined by gross examination of the carcass using criteria established by O'Gara (1978).

All samples were cultured on 5% sheep blood agar and eosin methylene blue agar (Remel, Lenexa, Kansas, USA) and incubated at 35–37 C for 24 hr. Bacterial colonies were screened to identify *Pasteurella* spp. based on typical colony morphology. Suspected *Pasteurella* spp. isolates were initially biochemically characterized by the API-20E (bioMerieux, St. Louis, Missouri, USA) system and those isolates refractory to a good identification were later retested using the Biolog System (Biolog, Incorporated, Hayward, California, USA). Isolates of *P. multocida* were serotyped for somatic antigens by the gel-diffusion precipitin test (Heddleston et al., 1972) and for capsule antigen by passive hemagglutination (Rimler and Brogden, 1986) at the U.S. Department of Agriculture (USDA), National Animal Disease Center (NADC), Ames, Iowa (USA). The isolates were compared to other serotype B:1 strains in the USDA, NADC culture collection by DNA fingerprinting. DNA fingerprinting using Hhal endonuclease was done as described by Wilson et al. (1992). Photographs of fingerprint profiles were scanned using a Hewlett-Packard IIcx flatbed scanner and Deskscanr software (Hewlett-Packard, Boise, Idaho, USA) and images were analyzed by the cluster analysis module of Gelcomparr software (Applied Maths, Kortrijk, Belgium) using the Dice coefficient. A dendrogram was derived from a matrix of similarity values by the unweighted pair group method using arithmetic averages.

Sixty-two percent (34 of 55) of the neonates examined were killed by coyotes (*Canis latrans*), 4% (2 of 55) died from septicemia due to *Pasteurella* spp. (both in 1997), and the remaining 34% died from a variety of causes including golden eagle (*Aquila chrysaetos*) predation, dystocia, starvation/nutritional deficiency, or unknown cause (Dunbar et al., 1999). We believe that the effects of capture and han-

dling on mortality of neonates were negligible because population surveys conducted in mid-July of each year found fawn:doe ratios very similar to those calculated from the survival of neonates in the study. Also, Byers (1977), in studies on handling pronghorn neonates in Montana, found no evidence that mortality risks were increased due to handling if proper precautions were taken, which we did in this study.

One neonate (#23) that died of septicemic pasteurellosis was an apparently healthy male captured at 2-days-old in May 1997 and later found dead at 17-days-old. The carcass was frozen for approximately 19 days and later thawed for necropsy. At necropsy, petechial and ecchymotic hemorrhages were found over a large portion of the subcutaneous tissues, skeletal muscle, epicardium, meninges of the brain and upon the serosal surface of the thoracic and abdominal cavities. A large amount of blood and foam was found in the bronchioles. The large intestine contained yellow mucoid feces.

Pathologic changes in pronghorn with pasteurellosis may include swollen and hemorrhagic nasal turbinates; pink froth in bronchioles; edema, congestion, consolidation, and hemorrhage in the lungs; enlarged lymph nodes; and widely scattered hemorrhages throughout the body (Thorne, 1982).

Histologic examination of the lung tissue of #23 revealed diffuse congestion and edema coupled with erythrocyte lysis and associated hemoglobin imbibition. Erythrocyte lysis and hemoglobin imbibition was apparently an artifact due to freezing. Frozen tissues precluded a complete histological examination. A moderate to marked multifocal infiltrate of macrophages, neutrophils, and numerous bacteria were found within many terminal bronchioles and alveoli.

Pasteurella multocida, serotype B:1 and *Pasteurella haemolytica* were isolated from the tonsillar crypt of #23. *Pasteurella multocida* also was isolated from the large intestinal tissues, lung, and thorasic fluid. The other neonate that died of septicemic pasteurellosis was an apparently healthy female (#18) captured when 2.5-hr-old in May 1997 and later found dead at 13-days-old. The carcass was refrigerated for approximately 4 days before examination. Gross lesions were similar to those of #23. Histologic analysis of tissues were not performed. *Pasteurella multocida*, serotype A:3,4 was isolated from the oropharyngeal area, heart blood, brain, lung, spleen, pericardial sac, cecal content, and thoracic fluid. A third neonate (#16) had gross lesions similar to those in #23 and #18, but *Pasteurella* spp. was not isolated and histological analysis was not performed. Therefore, a definitive diagnosis of pasteurellosis was not made. The carcass had been frozen for nearly 8 wk prior to examination.

The B serotype is reported to be rare in the U.S. (Wilson et al., 1995) and B:1 isolates have been obtained only from a very few domestic and wild species in the U.S. (Wilson et al., 1995). Serotype B:1 has not been reported as a cause of septicemic pasteurellosis in domestic species (Wilson et al., 1995), however a serotype B:1 *P. multocida* was isolated from two pronghorn in Montana, USA (Rhoades and Rimler, 1992) where its' role was not clearly detailed but assumed to be the cause of the mortality. Serotype A:3,4 has been reported previously to cause hemorrhagic septicemia in elk and deer (*Odocoileous* spp.) (Rimler et al., 1987; Carrigan et al., 1991), but is not normally considered the predominant strain for hemorrhagic septicemia compared to the B serotype (Wilson et al., 1995). Therefore, only the B:1 serotype was compared to fingerprint profiles of other species. The *P. multocida* B:1 isolate from pronghorn #23 had DNA restriction endonuclease fingerprint profiles (Fig. 1) distinct from isolates previously characterized including isolates from bovine, swan (*Olor* spp.), or moose (*Alces alces*) (U.S. Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, Iowa, USA,

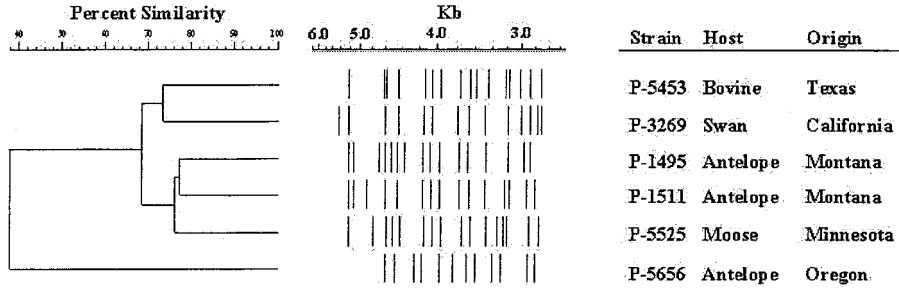

FIGURE 1. Comparisons of DNA fingerprint profiles of serotype B:1 *Pasteurella multocida* isolated from different host species.

unpubl.) (Fig. 1). Also, the two serotype B:1 *P. multocida* isolated from pronghorns (antelope) from Montana, reported by Rhoades and Rimler (1992), had different fingerprint profiles (Fig. 1) than B:1 isolates in this study.

Other pronghorns from which *Pasteurella* spp. were isolated in this study in 1997 but a definitive diagnosis of pasteurellosis was not made (n = 5;4 M, 1 F) included those found dead at 10 to 16-days-old and from which only a head and neck were recovered; these included animals that died, with no gross lesions of pasteurellosis, from an undetermined cause (n = 2) or were killed by a coyote (n = 3). From those, *P. haemolytica* was isolated from the tonsilar crypt (n = 1) and a mixture of *Pasteurella* spp./*Actinobacillus* spp. was isolated from tonsilar crypts (n = 4) and aqueous humor (n = 2).

Neonates that had *Pasteurella* spp. isolated from aqueous humor may have had septicemic pasteurellosis, however, because only the head and neck were recovered, no lesions suggestive of pasteurellosis could be identified. *Pasteurella* spp. were not isolated from neonates in 1996. This is the first reported case of septicemic pasteurellosis diagnosed in pronghorn from Oregon. Pasteurellosis is apparently a sporadic disease in pronghorns, and septicemia due to *Pasteurella* spp. has been reported only in Wyoming (USA)(Thorne, 1982) and Arizona (USA) (Powell, 1954). Beale and Smith (1973) found pneumonia in three fawns from Utah (USA) but no causative agent was isolated. Lance and Pojar (1984) reviewed diseases and parasites of pronghorn and *Pasteurella* spp. was not recognized as a cause of mortality in pronghorns.

Predisposition to pasteurellosis in pronghorns in this study may have included low (mean ± SD = 50.6 ± 15.1 ng/ml) whole blood selenium (Se) concentrations in neonates (n = 44) in 1997. There was a statistically significant difference ($P < 0.001$), using Student's t-test, between mean Se values from neonates in 1997, when pasteurellosis was observed, compared to Se values of neonates in 1996 (84.6 ± 20.6 ng/ml). The values in 1997 can be considered deficient if compared to serum Se values from deer (deficient, 7 to 60 ng/ml) (Puls, 1994). The blood concentration of Se known to produce clinical signs of deficiency have not been investigated in pronghorn. Cipriano et al. (1982), Larsen et al. (1988), and Finch and Turner (1989) have investigated the effects of supplementation of Se for domestic lambs and calves, and found that vitamin E and/or Se supplementation increased specific immune system responses during the early weeks of life, but the effect generally becomes less apparent in animals more than 6-wk-old. Although it is usually difficult to make interpretations across species of animals, unfortunately, these data are only available from investigations with other species, especially domestic animals.

Factors that may predispose pronghorns to pasteurellosis are not known but may

involve those associated with immature immune system in neonates, and other factors including poor nutrition such as low levels of trace minerals or vitamins. The significance of pasteurellosis to antelope populations is unknown and requires further investigation.

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