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Source: Journal of Wildlife Diseases, 24(4) : 663-667

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-24.4.663

Journal of Wildlife Diseases, 24(4), 1988, pp. 663–667

EXPERIMENTAL CONTACT TRANSMISSION OF *PASTEURELLA HAEMOLYTICA* FROM CLINICALLY NORMAL DOMESTIC SHEEP CAUSING PNEUMONIA IN ROCKY MOUNTAIN BIGHORN SHEEP

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ABSTRACT: Two Rocky Mountain bighorn lambs (*Ovis canadensis canadensis*) were held in captivity for 120 days before being housed with two domestic sheep. The lambs were clinically normal and had no *Pasteurella* spp. on nasal swab cultures. The domestic sheep were known to carry *Pasteurella haemolytica* biotype A in the nasal passages. After being in close contact for 19 days, *P. haemolytica* biotype A was cultured from nasal swabs of one of the bighorn lambs. By 26 days, both bighorn sheep developed coughs, were anorectic and became lethargic and nasal swabs yielded *P. haemolytica* biotype T, serotype 10. Twenty-nine days after contact, the lambs were necropsied and found to have extensive fibrinous bronchopneumonia. From affected tissues pure cultures of beta-hemolytic *P. haemolytica* biotype T, serotype 10 were grown. Both domestic sheep remained clinically normal and had no gross or microscopic lesions, but they carried the same *P. haemolytica* serotype in their tonsils. Behavioural observations gave no indication of stress in the bighorn lambs.

Key words: *Pasteurella haemolytica*, pneumonia, Rocky Mountain bighorn sheep, *Ovis canadensis canadensis*, experimental contact transmission, domestic sheep, experimental study.

INTRODUCTION

Respiratory disease is a serious factor in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) mortality and is responsible for major population reductions and subsequent slow population recovery (Marsh, 1938; Post, 1962; Stelfox, 1971; Spraker and Hibler, 1982; Onderka and Wishart, 1984). Bighorn sheep are susceptible to a variety of lung pathogens including viruses, bacteria and parasites (Post, 1962; Forrester, 1971; Clark et al., 1985; Dunbar et al., 1985). The interaction of these agents in the respiratory disease complex of bighorn sheep is still unknown; however, *Pasteurella haemolytica* is recognized to be associated with acute and chronic pneumonia often causing mortality in bighorns (Post, 1962) and domestic sheep (Gilmour, 1980). It has been suggested that bighorn sheep have a unique non-hemolytic strain of *P. haemolytica* that may be an opportunistic pathogen (Onderka and Wishart, 1984). However, in some epizootics, previous use of bighorn sheep habitat by domestic sheep or contact with domestic sheep has been incriminated as the source of infection (Foreyt and Jessup, 1982). This study describes experimental contact exposure of wild captive bighorn sheep to clinically normal domestic sheep in an attempt to demonstrate the possible transfer of *Pasteurella* spp. from domestic to bighorn sheep.

MATERIALS AND METHODS

Two male bighorn lambs, estimated to be born in late June and early July 1985 in the Sheep River Sanctuary in southwestern Alberta, Canada (50°40'N, 114°35'W) were captured in September using 80 mg xylazine (Haver-Lockhart Laboratories, Shawnee, Kansas 66201, USA) administered by projectile dart (Cap-Chur gun, Palmer Chemical and Equipment Co. Ltd., Douglasville, Georgia 30134, USA) and transported in individual crates to the research facility under xylazine sedation (4 mg/kg intramuscularly). They were housed individually on straw bedding for 30 days in well lit indoor stalls measuring 3.0 × 1.4 × 1.8 m at 15 to 20 C. This is in compliance with the 1980 guidelines of the Canadian Council on animal care (1105-151 Slater Street, Ottawa, Ontario, Canada K1P

5H3) and exceeds their recommendations for domestic sheep. The solid partition stalls were covered with chainlink wire and plywood to prevent the flight reaction of the sheep. They were each maintained on a ration of free choice good quality hay, 250 g commercial alfalfa pellets daily (Masterfeeds Division, Edmonton, Alberta, Canada T5B 4J3) and 2 g of iodized livestock salt (Masterfeeds Division) sprinkled on the hay every other day. Fresh water was supplied in secured 10 liter buckets. After this adaptation period of 30 days, the sheep were released together into a gravel floor chainlink fenced outdoor pen measuring 7 × 7 × 3 m. They were provided with an enclosed shelter 1.6 × 3.0 × 1.6 m attached to the pen. Here they were observed for 90 days for clinical signs of disease and their behavioural reactions were studied. They were readily caught and did not need constraint after a mask made from a toque was placed over their eyes. Their weights were taken every 2 wk. Twelve nasal swabs (Culturette, Marion Laboratories Inc., Kansas City, Missouri 64114, USA) were obtained for bacterial cultures during the 120 day observation period. Minimum overnight temperatures were recorded. On day 121, two clinically normal domestic 2-yr-old Suffolk ewes from the University of Alberta research farm (Edmonton, Alberta, Canada T6H 4P2) were added to the pen. The domestic sheep carried *P. haemolytica* in their nasal cavities as determined by cultures of nasal swabs. Subsequently, nasal swabs were obtained three times from all four sheep.

Bacterial cultures were plated directly onto blood agar consisting of tryptic soy agar base (Difco Laboratories, Detroit, Michigan 48232, USA) containing 5% sheep blood, MacConkey agar (Difco) and chocolate agar made from Proteose #3 agar base (Difco) containing 2% hemoglobin solution (Difco) and supplement B (Difco). Identification of *P. haemolytica* was made according to recognized characteristics (Weaver and Hollis, 1980; Mannheim, 1984). Further differentiation between biotype A and biotype T was done by the use of specific sugar reactions (Weaver and Hollis, 1980; Mannheim, 1984). All isolates were suspended in sheep blood and stored at −70 C. They were recultured onto blood agar plates and checked for purity. Selected colonies were picked, transferred to chocolate agar slants and sent to Dr. W. Donachie (Moredun Research Institute, 408 Gilmerton Rd., Edinburgh, EH17 7JH, Scotland), who confirmed the biotypes and provided the serotyping using indirect haemagglutination tests.

For virus isolations, lung tissue was ground in phosphate-buffered saline, centrifuged and the supernatant inoculated into 12th passage bighorn sheep fetal lung tissue culture cells grown in tissue culture flasks containing Eagle's minimum essential medium with 10% fetal calf serum (GIBCO Laboratories, Live Technologies Inc., Grand Island, New York 14072, USA). Cultures were incubated at 37 C and examined daily for 1 wk for cytopathic effects. The flasks were then frozen, thawed and reinoculated for 1 wk incubation.

Mycoplasma cultures from lung tissues were done by impression of a freshly cut tissue surface onto Hayflick's agar plates (Hayflick, 1965). In addition, lung tissue was emulsified in PPLO broth (Difco) inoculated into Hayflick's broth (Hayflick, 1965), incubated for 48 hr and replated onto Hayflick's agar plates. The replating was repeated in 48 hr and the cultures were held for observation for 10 days. Lung tissue was examined for bovine respiratory syncytial virus using the fluorescent antibody test on cryo sections with a conjugate from the Ministry of Agriculture, Fisheries and Food (Central Veterinary Laboratory, New Haw, Weybridge, Surrey KT15 3NB, England).

Sheep were euthanized using an overdose of Pentobarbital (M.T.C. Pharmaceuticals, Mississauga, Ontario, Canada L4W 2S3).

RESULTS

During the adaptation period the bighorn lambs showed no clinical signs of disease except for a transient mucoid nasal discharge on day 17 in the stalls. They slowly gained weight. Twelve nasal swabs taken periodically throughout the first 120 days yielded mixed scant to moderate growth of *Alcaligenes* spp., *Streptococcus* spp., *E. coli* and *Flavobacter* spp. They were negative for *Pasteurella* spp. On day 121, the two domestic sheep were introduced. Nasal swabs from the domestic sheep were previously cultured twice and yielded both times *P. haemolytica*, biotype A. No clinical signs were noticed. Within hours, the wild and domestic sheep intermingled calmly, fed together from the same heap of hay and drank from a common water source.

On day 19 after introduction of the domestic sheep, *P. haemolytica* biotype A was isolated from a nasal swab from one bighorn lamb. By day 26 after contact, both bighorn lambs started to shiver, were anorectic, developed coughs and soft stools, and became lethargic. Their rectal tem-

perature remained normal at 40 C. The minimum overnight temperature from day 19 to day 26 was −20 C. It had varied between −10 C and −30 C during the adaptation period. Coinciding with the onset of anorexia and lethargy was the isolation from nasal swabs of beta-hemolytic *P. haemolytica* biotype T from one bighorn lamb and again biotype A from the other. During the next 3 days, the clinical signs worsened to the extent that the animals remained recumbent when approached. Auscultation of the lungs revealed rasping rales over the anterior thorax. Lateral radiographs showed extensive water density of most of the ventral lung fields obscuring the heart silhouette.

On day 29 and day 30 after exposure to the domestic sheep, the bighorn lambs were euthanized. Necropsy showed both animals to be well muscled and to have ample body fat. Tonsils and retropharyngeal lymph nodes were enlarged. The anterior ventral lung lobes were enlarged, firm and dark red. The pleura was covered by a thin film of fibrin. In one lamb, the anterior lobe of the lung had multiple small areas of necrosis. The middle lobes and the anterior diaphragmatic lobes had more acute lesions and were red, heavy and rubbery with areas of hemorrhage. Both lambs had few small, grey, firm nodules in the posterior margin of the diaphragmatic lobes. These were confirmed histologically to be due to lungworm infection (*Protostrongylus* spp.) with adult nematodes, eggs and larvae. Bronchial lymph nodes were enlarged. Microscopic examination of the lung lesions confirmed a necrotizing fibrinopurulent bronchopneumonia. There were discrete areas of coagulation necrosis delineated by degenerated, inflammatory cells. Alveoli contained proteinaceous edema fluid. Fibrin accumulated on the pleura as well as in interlobular septae.

Based on characteristics described in cattle (Friend et al., 1977; Allan et al., 1985), the lesions we observed in the bighorn lambs were estimated to be about 3 days old. *Pasteurella haemolytica* biotype T, serotype 10 was isolated from the lungs, tonsils, bronchial lymph nodes and pharyngeal swabs of both lambs; their kidneys and spleens were negative. Virus isolation attempts were negative. Fluorescent antibody tests for bovine respiratory syncytial virus were also negative. Mycoplasma cultures from lungs, bronchial and pharyngeal lymph nodes showed no growth.

The domestic sheep remained asymptomatic throughout the study period and were euthanized the day after the second bighorn was euthanized. They were in excellent body condition and had no visible gross or microscopic lesions in tonsils, bronchial lymph nodes or lungs. Bacterial cultures from nasal swabs again yielded *P. haemolytica* biotype A. Cultures of tonsils from both domestic sheep contained *P. haemolytica* biotype T, serotype 10.

DISCUSSION

For many years, wildlife biologists and researchers have suspected that even casual contact between bighorn sheep and domestic sheep may lead to respiratory disease with fatal pneumonia in the bighorns. Transfer of pathogens was suggested although the organisms involved could not be documented.

Our study of a major bighorn sheep epizootic in southern Alberta in 1981–1982 (Onderka and Wishart, 1984) suggested that *P. haemolytica* was a significant agent in the development of pneumonia, but with no evidence that a virus, *Mycoplasma* spp., or *Chlamydia* spp. was involved. All of the collected sick or dead sheep had fibrinopurulent bronchopneumonia. Bacterial cultures from chronically infected tissue gave a mixed growth of *Acinetobacter* spp., *Corynebacterium* spp., *Flavobacter* spp., *Pseudomonas* spp. and *E. coli*. From acute lesions a pure growth of a non-hemolytic variant of *P. haemolytica* (Onderka et al., 1988) was isolated from 40% of 30 sheep sampled. In addition, from 7% of the sheep, either a beta-hemolytic biotype T, biotype A or both biotypes were isolated. A sub-

sequent survey using nasal and pharyngeal swabs from 240 apparently healthy bighorns yielded no *Pasteurella* spp. except from one lamb whose ewe had chronic bronchopneumonia and had been culled previously from the herd. These sheep were sampled from various areas of the Rocky Mountains in Alberta. They were hunter submissions of ewe heads and lungs, accidental deaths and sheep caught at bait stations.

Based on reports that *Pasteurella* spp. may be dormant in tonsils in domestic sheep (Gilmour et al., 1974; Al-Sultan and Aitken, 1985) a second survey was done using cultures from cut sections of tonsils. This time, 25% of 61 bighorn sheep had the non-hemolytic variant of *P. haemolytica* biotype T including the serotypes 3, 4 and 15. A survey of tonsils taken at slaughter from 40 domestic sheep originating from nine different areas in Alberta and British Columbia showed only beta-hemolytic strains of both biotypes, but mainly biotype A.

Based on these data, the domestic sheep used in the present study were chosen to be infected with *P. haemolytica* biotype A which would be easily differentiated from the "bighorn strain." The bighorn lambs were observed for 120 days without showing any clinical signs of disease despite confinement and very low environmental temperatures. After the introduction of domestic sheep, sudden onset of respiratory disease leading to extensive bacterial pneumonia occurred in the bighorns within 26 days. The intimate contact between the two species of sheep in this experimental design rarely occurs in the wild except when young bighorn ram seek out domestic ewes. Close contact, however, was necessary for the possible transfer of *Pasteurella* spp. since this organism does not persist in the environment (Blood et al., 1983).

The bighorn lambs in the present study developed severe pasteurella pneumonia with no evidence of virus or *Mycoplasma* spp. involvement. The tissues were not cultured for *Chlamydia* spp. which could possibly have predisposed the sheep to other microorganisms but histologically the lung lesions were not suggestive of enzootic pneumonia. The lambs may have harboured *Pasteurella* spp. organisms in their tonsils where they might have proliferated if the lambs were stressed by the presence of domestic sheep. However, daily observations showed no behavioural evidence of stress. There were no dominance fights and food was supplied so that all four sheep could feed at the same time.

After exposure to domestic sheep, nasal cultures of one of the bighorn lambs which has been consistently negative for *Pasteurella* spp. yielded *P. haemolytica* biotype A. Although the tonsils of the bighorn lambs were not incised and cultured at the onset of the study, there is evidence of a nose to nose transmission of *P. haemolytica* biotype A. The pneumonic lesions in the bighorn lambs were associated with beta-hemolytic *P. haemolytica* biotype T, serotype 10 which also was recovered from the tonsils of the domestic sheep.

The recovery of a specific serotype not commonly found in bighorns also suggests that a transfer of *P. haemolytica* may have occurred. It should be emphasized that no particular importance or pathogenicity of *P. haemolytica* biotype T, serotype 10 is implied, but that its identification merely served to trace possible transmission. Because of the consistent cultural and biochemical characteristics of the non-hemolytic pasteurella strain of bighorns (Ondérka et al., 1988), it is suggested that the 1981–1982 epizootic in bighorn sheep in southern Alberta probably did not originate from pathogen transmission between domestic sheep and bighorns.

ACKNOWLEDGMENTS

We thank Bernise Fougere-Tower for her outstanding dedication to the care and maintenance of bighorn sheep. We thank Shirley Rawluk for her excellent bacterial culture work, Marco Festa-Bianchet and Jon Jorgenson for capture and transport of the lambs and the An-

imal Science Department, University of Alberta for providing the domestic sheep.

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Received for publication 5 January 1988.