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PHARYNGEAL MICROFLORA OF DALL AND DOMESTIC SHEEP IN ALASKA: MANAGEMENT IMPLICATIONS?

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Abstract: Anticipating the occurrence of disease-related, all-age die-offs is a common component of contemporary bighorn management strategies. Domestic sheep have often been implicated in these bighorn die-offs. In Alaska, documented exposure of Dall sheep to domestic sheep has been limited to 2 3-to 6-week periods during late-winter when domestic sheep were run on a Dall sheep winter range near Palmer. The results of this exposure are not yet known, but a disease-related die-off in Dall sheep has never been documented. As the exposure of Dall sheep to domestic sheep near Palmer demonstrates, wildlife managers in Alaska were unable to exclude domestic sheep from Dall sheep ranges. Because data demonstrating effects of domestic sheep diseases on Dall sheep are not available, we began to investigate a possible causative relationship between domestic sheep and disease in Dall sheep. We started with a bacteriologic survey of tonsils from 16 domestic ewes produced in the Tanana Valley near Fairbanks. These ewes carried pharyngeal microflora typical of those reported for domestic sheep elsewhere, including the Pasteurella haemolytica strains and serotypes which have been implicated in bighorn die-offs. Next, 43 wild Dall sheep of both sexes from a remote area of the Brooks Range were also assayed for pharyngeal flora using tonsil biopsies and nasal swabs. These Dall sheep carried numerous species of bacteria, including the P. haemolytica strains and biotypes implicated in bighorn sheep die-offs. Relevance of these findings to the domestic-bighorn sheep connection and future research possibilities are discussed.

Anticipating occurrence of disease-related die-offs of bighorn sheep is a common component of bighorn sheep management strategies in North America (Heimer 1990). The literature which necessitates this management position is extensive. After description of the lungworm (Protostrogylus stilesi) life cycle came the demonstration of alternate-host "hot spots" (Lange 1974), and the demonstration of transplacental transmission of lungworm larvae (Schmidt et al. 1979). These findings led most bighorn managers to conclude that the lungworm-pneumonia complex was a classic density-dependent factor limiting bighorn populations. With the development and administration of anthelmintic

drugs (Schmidt et al. 1979, Foreyt and Johnson 1980), it became possible to mitigate some of these effects; and many managers thought the disease problem had been solved.

These findings had scarcely been presented when a major, well documented die-off of bighorn sheep began in the Kootenay region of British Columbia. This die-off was the best-studied large disease event in the history of bighorn sheep. The results implicated P. haemolytica varieties as etiologic agents (Onderka and Wishart 1984, Schjwantje 1984).

In classically simple experiments, Onderka and Wishart (1988) demonstrated that this fatal pneumonia could be produced in healthy bighorn sheep by P. haemolytica nonhemolytic biotype T cultured from the tonsils of bighorns that died during the epizootic. Onderka also demonstrated this bacterium, cultured from healthy domestic sheep, caused fatal pneumonia in captive bighorns. Additionally, Onderka (1986) produced fatal pneumonia among captive bighorns with biotype A of this Pasteurella spp., which had been modified for use as a cattle vaccine. These experiments also demonstrated a predisposing viral infection was unnecessary for development of fatal bacterial pneumonia in captive bighorns. Domestic sheep were consistently and uniformly refractory to the organisms which killed bighorns.

Domestic sheep were further implicated as carriers of pathogens for bighorns by the work of Foreyt (1988, 1990). In replicate trials, he showed that apparently healthy bighorn sheep soon perished when apparently healthy domestic sheep were introduced into their pens. Callan et al. (1991) also demonstrated fatal pneumonia among desert bighorns penned with domestic sheep. Again, Pasteurella spp. was implicated. These findings clearly suggest that allowing domestic sheep on wild bighorn ranges carries some risk.

In Alaska, exposure of Dall sheep to domestic sheep was undocumented until domestic sheep were found on Dall sheep winter ranges between the Knik and Matanuska Rivers near Palmer, Alaska in spring 1990. These domestic sheep mingled with Dall sheep on grazing leases let by the Chickaloon Native Association (CNA). CNA had received the land as part of the settlement of their aboriginal land claims (Heimer 1980), and leased grazing on it to generate income. These unfenced grazing leases were adjacent to late-winter ranges used by Dall sheep. Immediately after snow at lower elevations melted in spring of 1990, domestic sheep were introduced to the unfenced grazing leases by the lease holder. There, they mingled with Dall sheep on their late-winter ranges for several weeks before the Dall sheep moved to summer ranges. The domestic sheep remained alone on the Dall sheep winter ranges until fall 1990 when they were moved to wintering grounds away from Dall sheep ranges. In early spring 1991, the domestic sheep were again returned to Dall sheep ranges where they once again mingled with Dall sheep until the Dall sheep again left for their summer ranges. After the domestic sheep were removed in fall 1991, they were sold, and not returned to the lease.

Although we were surprised by this specific introduction of domestic sheep to Dall sheep range, Alaskan Dall sheep and wildlife disease researchers had already identified potential problems with introduction of diseases by domestic livestock (Heimer et al. 1982). As a result of our earlier concern, we continued to document the disease histories of Dall sheep throughout Alaska. Our previous work centered on viral serology (Zarnke et al. 1983; Zarnke 1987, 1991; Zarnke and Rosendal 1989).

As a response to demonstration that fatal bacterial pneumonia could occur in bighorn sheep independent of lungworm or viral infection, we expanded our program to include bacterial pathogens. Bacterial diseases, particularly pneumonia involving Pasteurella spp., were of greatest interest because of the findings listed above. Bacterial investigations of both domestic and Dall sheep in Alaska became practical when it was reported bacteria could be isolated from the tonsils of wild and domestic sheep under Alaskan field conditions (D. Onderka, Univ. Alberta, pers. commun.; Dunbar et al. 1990).

METHODS

We slaughtered 16 adult mixed-breed domestic ewes which had been produced and maintained in the Tanana Valley in Interior Alaska. Prior to slaughter, nostrils were swabbed with rayon-tipped swabs. Swabs were introduced into Amies modified medium with charcoal. Specimens were stored for 36 hours at 25 C, then packaged in insulated cool containers, and shipped by same-day mail to Al Ward of the Caine Veterinary Center, Caldwell, Idaho where they were immediately plated and subsequently identified (Queen et al. 1992).

At slaughter, the soft palates of these domestic ewes, including the tonsilar crypts, were removed and packaged in plastic bags. Samples were then air-expressed to Dr. Ward where he inoculated cultures within 48 hours. See Queen et al. (1992) for details.

Dall sheep from the south slope of the eastern Brooks Range were captured by helicopter using a skid-mounted projectile net (Heimer and Mauer 1990). Nasal swabs and tonsil biopsies (Dunbar et al. 1990) were collected from 19 sheep in October 1990. An additional 9 swab and biopsy samples were collected from Dall sheep in the same area in 1991. Fifteen sample sets were also collected from the north side of the Brooks Range in the Hula Hula River drainage at this time. All samples were flown to Arctic Village and air-expressed to bacterial laboratories identification and typing.

RESULTS

Domestic sheep produced in Alaska harbored pharyngeal microflora typical of domestic sheep in the lower 48 states and Canada. This included the Pasteurella spp. organisms which have been implicated in wild sheep die-offs. Details of the bacterial species found in the tonsils of domestic sheep in Interior Alaska were reported by Queen et al. (1992). Six gram-negative bacteria were present in notable percentages (Table 1).

Table 1. Gram-negative bacteria present in 16 adult domestic ewes from the Tanana Valley, Alaska in summer, 1990.

Bacteria	Occurrence (%) Nasal	Tonsil
Enterobacter agglomerans	64	45
Enterobacter cloaca	45	0
Neisseria denitrificans	0	64
Moraxella ovis	0	18
Pasteurella haemolytica T	0	91
Pasteurella haemolytica A	0	27

Dall sheep from the remote portions of the Brooks Range, had pharyngeal microflora which included P. haemolytica biotypes A 5, A-untypable, T 3, T 4, T 10, and T-untypable (Table 2). Pasteurella spp. organisms were not isolated from material collected in 1991. Delay in getting the material to the laboratory was the probable cause of these negative results.

DISCUSSION

There are currently 3 schools of thought regarding the origin of Pasteurella spp. pneumonia in bighorn sheep. One school, led by Foreyt, suggests contact with domestic sheep infects bighorns with a fatal Pasteurella spp., which is foreign to them, but is carried by domestic sheep without effects. Once bighorns are infected, high mortality results (Foreyt 1990). Prevention of exposure by keeping domestics and bighorns apart is seen as unlikely for political and economic reasons, so treatment by development of a vaccine and vaccinating bighorns has been recommended by this school.

The second school suggests bighorn sheep typically carry the pathogenic Pasteurella spp. strains and biotypes, and that stress compromises the bighorn immune system resulting in fatal pneumonia (T. Spraker, Colo. State Univ., pers. commun.; Belden et al. 1990) . We infer that the management recommendation of this school is to limit stresses to benefit bighorn populations.

The third school suggests there are both bighorn and domestic varieties of Pasteurella spp.. Should bighorns become infected with the domestic strain, they will develop pneumonia and die, but domestic sheep are not affected by the bighorn strain (Onderka and Wishart 1988). We infer the recommendation of this school is to prevent contact between domestic sheep and bighorns. If separation of bighorns from domestics is not maintainable, bighorns should be managed as wild sheep to minimize losses to disease, but not vaccinated or treated (W. Wishart, Alberta Wildlife Branch, pers. commun.).

Table 2. Bacteria isolated from nasal swab and tonsil biopsies of 19 Dall sheep from the Brooks Range, Alaska in 1990.

Bacteria	Positive isolates	
	Nasal	Tonsil
Pseudomonas flourescens	2	3
P. solanacearum	1	0
P. syringae PV aptata	0	1
P. sp.	1	0
Flavobacterium meningosepticum	1	0
Moraxella bovis	2	0
Moraxella osloensis	1	1
Moraxella sp.	0	1
Actinobacillus actinomycetem comitans	2	0
Actinobacillus sp.	1	0
Pasteurella haemolytica T 3, T 4, T 10	0	6
Pasteurella haemolytica T-untypable	0	1
Pasteurella haemolytica A 5	0	1
Pasteurella haemolytica A-untypable	1	1
Pasteurella-like	4	3
Eschericia coli	1	8
Acinetobacter calcoaceticus	1	0
Enterobacter agglomerans	0	1
Micrococcus sp.	1	0
Staphylococcus aureus	1	2
S. epidermidis	0	3
S. haemolyticus	1	0
S. homins 2	0	1
S. saprophyticus	1	0
S. sciuri	1	0
S. typhi A	0	1
S. warneri	2	3
S. xylosus	3	1
Streptococcus bovis 1	1	2
S. bovis 2	3	2
S. bovis 1 or 2	1	3
S. equisimilis	1	0
S. mitis	0	1
S. moribillorum	0	1
S. mutans	2	1
S. salivarius	0	1
S. sanguis I	0	2
S. sanguis 3	1	0
S. suis 1	2	0
S. spp.	1	2
Bacillus spp.	2	2

Dall sheep in the remote parts of the Brooks Range have, in all probability, never been in contact with domestic sheep. Still, these Dall sheep carry P. haemolytica biotypes A 5, T 3, T 4, and T 10, as well as untypable varieties of A and T. These are presumably the same Pasteurella spp. biotypes which produce fatal pneumonia in bighorns, yet no disease-related die-offs have been documented in Dall sheep. These findings, along with the commonly accepted origin of North American wild sheep, a common ancestor which migrated from Asia via Beringia (Cowan 1940, Geist 1971), suggest Pasteurella spp. bacteria are enzootic to wild North American, and perhaps Asian, mountain sheep. If this is so, it is probable that North American wild sheep have "always" had these bacteria. Hence, it is unnecessary to postulate domestic sheep as the source of all Pasteurella spp. bacteria which have been implicated in bighorn pneumonia. Furthermore, apparently healthy bighorns, both in pens and in the wild, carry Pasteurella spp. bacteria (T. Spraker, Colo. State Univ., pers. commun.). Our conclusion, that the pneumonia pathogens need not come entirely from domestic sheep, is based on these data and the assumption that conventional laboratory techniques (Ward et al. 1990) characterize these bacteria adequately.

Still, we question whether conventional laboratory techniques adequately characterize these bacteria. Pasteurella spp. is one of several gram-negative, pleomorphic bacteria (Ward et al. 1990). Pleomorphic bacteria change their form, or morphology, depending on culture media. If the morphology of these bacteria is variable, we suggest it is possible that ability to use specific metabolites, which is the basis of conventional taxonomy, may change also. Should changes in metabolic capability be associated with morphological changes, they would produce differing results in biotyping and perhaps even species identification. If this were the case, conventional classification on the basis of Gram-staining and metabolite use in culture would result in differing designations for a single organism which is more adaptable than we presently appreciate. Techniques beyond metabolic capability in culture may be required to address these questions.

The finding that Dall sheep have pharyngeal microflora which are quite similar to those of domestic sheep does not contradict the stress or species-specific schools of thought. However, it is difficult to imagine that Dall sheep exist with less stress than commonly defined for bighorn sheep. Most Dall sheep have lungworms. Those which have been studied appear to have moderate larval fecal outputs of 200 larvae per gram of feces. Additionally, Dall sheep endure at least 8 months of winter each year, are subject to natural predation, human harvest, intense non-consumptive use, and industrial development. Finally, Dall sheep frequently exist at densities which are greater than bighorn populations which have suffered die-offs in the past. Still, we do not see all age die-offs among Dall sheep.

Through the process of elimination, the Onderka and Wishart hypothesis seems most acceptable for describing the origin and pathogenicity of P. hemolytica. Without better identification of these bacterial varieties, we are unable to offer a better explanation. Several laboratories are working on the nuclide sequences from the

Pasteurella spp. organisms isolated from Dall and domestic sheep from Alaska as well as from bighorn and domestic sheep from other regions. We are currently unaware of their findings.

The possibility that Dall sheep are immunologically more competent than bighorn sheep also exists. If specialization, which Geist (1971) postulated for bighorn races, resulted in a narrower spectrum of immune responses, it would be reasonable to anticipate more robust immunocompetence in the less specialized thinhorn races.

These possibilities could be addressed experimentally by inoculating Dall sheep with various Pasteurella spp. organisms from domestic and bighorn sheep under well-controlled laboratory conditions. Alternately, comparative studies of Pasteurella spp. cytotoxins on Dall and bighorn sheep white blood cells may be useful. We plan such experiments in the future. We will cooperate by supplying Dall sheep or Pasteurella spp. organisms from Dall sheep to investigators who present well designed experiments to test these possibilities. In any case, we suggest that Dall sheep with no history of involvement with domestic sheep present good experimental opportunities to further investigate the development of Pasteurella spp. pneumonia pending the outcome of DNA sequence analysis.

Whatever the cause of Pasteurella spp.-related die-offs, history shows that prevention of disease is more effective than treating it. Consequently, our recommendations are:

1. Wherever possible, prevent domestic and wild sheep mixing.

   In Alaska, the Alaska Department of Fish and Game (ADF&G) will continue to oppose introduction of domestic livestock, especially domestic sheep, to Dall sheep ranges.

   Close cooperation between ADF&G and the Foundation for North American Wild Sheep recently resulted in removal of domestic sheep from Dall sheep range near Palmer, Alaska. Sampling of sheep from the domestic and wild sheep herds involved in this transient exposure is a high priority. Management actions appropriate to the findings will be recommended.

2. Wherever possible, minimize stresses on mountain sheep populations.

   Dall sheep appear, by our standards, to be highly stressed. Still, most of the stresses we can identify are stresses to which they have become adapted over time. Introduction of new stressors should be carefully weighed and mitigated to the extent possible.

3. Accelerate work on DNA sequencing of Pasteurella spp. and other Gram-negative pleomorphic bacteria.

4. Address the question of differing levels of immunocompetence among bighorn and thinhorn races of wild sheep.

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