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NATURALLY OCCURRING PNEUMONIA IN CAESARIAN-DERIVED ROCKY MOUNTAIN BIGHORN SHEEP LAMBS

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Abstract: Survival of bighorn lambs beyond 10 weeks of age was reported to be as low as 4% in some regions of central Idaho in 1988-89. Observations of lambs during the first weeks of life in 1990 confirmed early death losses but did not establish the causes. Pregnant ewes were captured in April of 1991 from 2 herds in which low lamb recruitment had been reported. Single male lambs were taken by caesarian-section from 2 of the ewes and a third ewe gave natural birth to a single ewe lamb. The caesarian-derived lambs were bottle fed in isolation for the first 3 weeks of life and remained free of Pasteurella spp. until they were exposed to their mothers. At 8 and 10 weeks of age the 2 lambs developed evidence of inner ear irritation and pneumonia. The infections were allowed to progress until it was concluded that the lambs would die due to the pneumonia complex if left untreated. Transtracheal wash samples were collected from the lungs of each lamb. Lungworms (Protostrongylus stilesi), transmitted from the dams by the placenta, and biotype T P. haemolytica were recovered from transtracheal wash samples of both lambs. Both lambs recovered fully after treatment with antibiotics and an antihelmintic.

Idaho Department of Fish and Game officials, backpackers and hunters reported hearing harsh coughing from the majority of bighorn sheep (Ovis canadensis canadensis) observed on the Salmon River drainage of central Idaho in fall and winter 1988-89. Survival of lambs was subsequently reported to be low in herds of that area in 1989 and 1990. Respiratory disease has been reported to be a primary factor associated with poor lamb survival (Spraker et al. 1984). Potential causes of respiratory disease are multiple and may vary between herds, climatic conditions, and other contributing factors. Viruses, bacteria, and lungworms have been identified as causes of pneumonia associated with lamb mortality (Parks and England 1974, Spraker et al. 1986). University of Idaho, Department of Fish and Game, and Idaho Department of Agriculture personnel joined in efforts to identify causes of respiratory disease and poor lamb survival in Idaho herds.

During spring and summer 1990, 10 radio-collared ewes were monitored to determine the health status of their lambs (J. and H. Akenson, pers. commun.). Each of the 10 ewes delivered live lambs, all of which died within 8 weeks of birth. Three lamb carcasses were found and examined for causes of death. However, scavenger activity and the small number of carcasses found limited the amount of information that could be applied to determination of causes of death of the 10 lambs. Therefore, the following study was conducted to evaluate the health of lambs in a controlled environment.

METHODS

Two 4-year-old ewes were captured on 11 April 1991 by helicopter net-gunning techniques from free-ranging herds near Challis, Idaho on the Morgan Creek drainage. They were hobbled, blind-folded, and transported by helicopter in sheep-bags from the capture site to a processing location. Blood, feces, tonsillar, and nasal samples were collected from the ewes while physical and pregnancy examinations were made. Body temperatures of the ewes were closely monitored to avoid hyperthermia. The ewes were loaded into a 1.3 X 2.5 m darkened wood box and transported approximately 358 km (222 mi) to a 30.3 X 30.3 m pen near the University of Idaho Caine Veterinary Teaching and Research Center (CVTRC) south of Caldwell, Idaho. A third ewe was captured on 8 April 1991 on the Big Creek drainage east of McCall, Idaho by immobilization with 3 mg carfentanil citrate injected with a dart gun. This ewe was hobbled, blind-folded, and injected with 300 mg naloxone. Half of the naloxone was injected intramuscularly and the remainder subcutaneously into the ewe prior to her transport by airplane to the Caldwell airport. She was then taken by truck to the Caldwell facilities and placed in a pen 15 m from the pen for the ewes from Morgan Creek. The pens were constructed of wood poles 20-30 cm in diameter, 1.85 m high New Zealand fencing and topped with 3 strands of high tensile wire which was connected to an electrical fence unit. Black depredation netting was attached to the inside of the New Zealand fencing to make it more visible for the sheep. The ground level on the eastern side of the pen was approximately 3 m higher than on the west side thus providing an area where the sheep could seek higher elevation and reduce stress associated with the presence of people presenting feed at a lower gate. Mixed grass and alfalfa hay was placed in the lower portion of the pen once a day and water was provided in an automatic waterer. The animals were watched with field glasses from a distance of approximately 100 m to monitor mammary development signalling approaching parturition.

On 16 May when it was judged that the ewes were close to lambing, they were captured by use of linear drive nets, blind-folded, hobbled, and transported to the CVTRC surgical section. The ewes were prepared for abdominal ultra-sonogram (Aloka 210 instrument; Corometrics, Wallingford, Connecticut 06492 USA) and checked for colostral secretions. Both of the Morgan Creek ewes were producing colostrum and ultra-sonograms indicated well developed lambs. The udder of the Big Creek ewe contained minimal secretions and the ultra-sonogram of the lamb indicated a less developed lamb. Therefore, the decision was made to take the lambs by caesarian-section from the highly excitable Morgan Creek ewes, but not from the more

docile Big Creek ewe. Anesthesia of the ewes was induced and maintained with halothane. Caesarian-sections were performed through a ventral midline surgical approach. Both lambs (BR91-017 and BR91-018) were males and judged to be near term. Colostral secretions were collected from the ewes and given to their respective lambs, both of which nursed vigorously from bottles. Surgical sites were closed and the ewes were returned to their enclosure. Antibiotics were not administered postoperatively. Recovery of the ewes was complete and unremarkable. The third ewe gave birth to a ewe lamb (BR01-019) 1 week after caesarian-sections were performed on the 2 other ewes.

The caesarian-derived lambs were isolated from other animals and fed non-sweetened canned evaporated milk every 4-6 hrs for the first 2 weeks of life. At 15 days postdelivery (PD) feeding of pasteurized milk from domestic ewes was initiated. A lamb milk replacer (Land O'Lake, Inc., Fort Dodge, Iowa 50501 USA) was used to supplement ewe milk as needed through the 7th week. Milk replacer was the only source of milk fed during weeks 8-18. Rectal body temperatures were taken at each feeding time. Free access to mixed grass and alfalfa hay and water was provided following the 2nd week. The lambs were placed in pens with their mothers for 1-4 hrs/day on PD days 20, 22, 28, 29, 34, 35, 37 and 44 to allow them to acquire microbial flora from their dams.

Culturette swabs (Marion Laboratories, Inc., Kansas City, Missouri 64114 USA) were used to collect nasal and tonsillar samples from the ewes and lambs. These samples and transtracheal wash samples were cultured for bacteria at CVTRC. Viral transport swabs (Becton Dickinson Microbiology Systems, Cockeysville, Maryland 21030 USA) were used to collect samples from ewes and lambs. The latter swabs were submitted to the Washington State Animal Disease Diagnostic Laboratory (WADDL) for virus isolation procedures. Fecal samples were collected from ewes at the time of capture and monthly for 2 months, from the caesarian derived lambs at 12 days, and from all lambs at approximately 1 month of age. All fecal samples were examined for parasites at CVTRC. In addition, sera were submitted to the Idaho State Animal Industries Laboratory for testing to detect antibodies against viruses and bacteria (Table 1).

Three media; Columbia blood agar with 5% ovine blood (CBA), Columbia blood agar with bovine blood plus antibiotics to provide selectivity for Pasteurella (Ward et al. 1986), and Hayflick's agar selective for Mycoplasma (Stalheim 1990) were inoculated for isolation of bacteria. Culture media were incubated at 35C in an atmosphere with 5% added CO_2. All bacterial isolates were evaluated. Pasteurella identification and biotype differentiation were conducted by established procedures (Carter 1990, Kilian and Frederiksen 1981). Serotyping of the Pasteurella haemolytica isolates was conducted by slide agglutination tests with specific antisera (Frank and Wessman 1978).

RESULTS

All ewes were clinically normal prior to and following delivery of their lambs. The naturally delivered ewe lamb BR91-019, was vigorous and did not demonstrate clinical illness during the 5-month observation

Table 1. Antibody titers in sera of adult bighorn ewes to bacterial and viral pathogens, Caldwell, Idaho.

Disease agents (tests)[a]	Antibody titers/animal BR91-003	BR91-008	BR91-012
Bacterial:			
Anaplasma marginale (CF)	10[b]	NEG	NEG
Brucella ovis (ELISA)	NEG	NEG	NEG
Campylobacter fetus venerealis (MA)	NEG	NEG	NEG
Haemophilus somnus (MA)	NEG	NEG	NEG
Leptospira interrogans (MicroA)	50	NEG	50
Viral:			
Bluetongue virus (AGID)	NEG	NEG	NEG
Bovine viral diarrhea virus (SN)	8	NEG	NEG
Epizootic hemorrhagic virus (AGID)	NEG	NEG	NEG
Infectious bovine rhinotracheititis virus (SN)	8	8	NEG
Ovine progressive pneumonia virus	NEG	NEG	NEG
Parainfluenza-3 virus (SN)	NEG	NEG	NEG
Respiratory syncytial virus (SN)	NEG	8	8

[a]Antibody titers were quantitated by: CF, complement fixation procedure; ELISA, Enzyme-linked immunosorbent assay; MA, macroscopic agglutination; MicroA, microscopic agglutination; AGID, agar gel immunodiffusion; SN, serum neutralization.

[b]A titer of 10 to Anaplasma marginale antigen is considered significant. All other listed titers are too low to be considered diagnostic.

period. At 50 days PD, lamb BR91-018 developed ear irritation as demonstrated by tilting of the head to the right, drooping of the right ear, scratching of the ear with his rear leg, and head shaking. Otoscopy revealed a reddened ear-drum but no evidence of external irritants. On day 52 the lamb coughed frequently and the rectal temperature was elevated to 40.2C. The temperature continued to increase to 41.5C by day 54. The lamb reduced his milk intake, had signs of dehydration, depression, dyspnea, and was reluctant to move on day 55 when the decision was made to collect a transtracheal wash sample and initiate antibiotic treatment. An initial injection of Durapen™ (VEDCO, Inc., Overland Park, Kansas 66204 USA) was given followed by 6 daily injections of ampicillin. Both lambs coughed easily when pressure was applied to their chests. However, Lamb BR91-017 did not demonstrate clinical illness until day 65 when a head tilt and drooping of both ears became evident. The latter lamb was febrile (39.8C) on PD day 70 and was reluctant to nurse a bottle. The lamb was depressed by the following day when the rectal temperature was 40.6C. Therefore, a transtracheal wash sample was collected for examination. Two days later it appeared the lamb was becoming progressively worse and death appeared imminent. Lamb BR91-018 also continued to cough and appear slightly depressed. Therefore, both lambs were given daily injections of Naxcel™ (The Upjohn Company, Kalamazoo, Michigan 49001 USA) for 2 days followed by amoxicillin for 5 days. Ivermectin (MSD AGVET, Division of Merck & Co. Inc., Rahway, New Jersey 07065 USA) was given to both lambs on day 76 for elimination of Protostrongylus spp.

Pasteurella spp. were not isolated from nasal and tonsillar swab samples taken from the caesarian-derived lambs until they were 40 days old and had been placed in the pens with their mothers on repeated occasions (Table 2). Biotype A isolates were recovered from tonsillar samples of both caesarian-derived lambs when they were sampled at 40 days PD. At day 53, the predominant organisms in samples from these animals was P. haemolytica biotype T which agglutinated in antisera for types 3, 4, and 10. Biotype T P. haemolytica was isolated in pure culture and high numbers from the transtracheal wash samples. Pasteurella haemolytica biotypes A and/or 3 were isolated from all samples collected from lamb BR91-019 beginning at 10 days after birth. Biotype T P. haemolytica was not isolated from any samples collected from either this lamb or her dam.

Pasteurella haemolytica biotypes T and 3, and P. multocida were isolated from samples collected from the ewes (Table 3). The majority of P. haemolytica biotype T isolates agglutinated in antisera 3, 4, and 10. However, 1 biotype T isolate agglutinated only in antiserum for serotype 10.

No parasites or ova were detected in any of the first fecal samples from the lambs. However, P. stilesi was detected in the transtracheal wash samples from the 2 caesarian-derived lambs but not from the other lamb. Lungworm larvae were detected in the feces of the ewes from both Morgan Creek and Big Creek areas. In addition coccidia, Trichuris and Nematodirus were detected in feces of the ewes.

Table 2. *Pasteurella* cultured from samples collected from bighorn lambs at indicated ages, Caldwell, Idaho.

Lamb age (days)	Lamb ID No.	Type of sample[a]	*Pasteurella* isolated
10	BR91-019	N	none
		T	*P. haem* A[b] (UT)[c]
			P. haem 3
12	BR91-017	N, T	none
	BR91-018	N, T	none
29	BR91-019	N	*P. haem* A (1,2)
		T	*P. haem* A (UT)
			P. haem 3
34	BR91-017	N, T	none
	BR91-018	N, T	none
38	BR91-019	N	none
		T	*P. haem* 3
40	BR91-017	N	none
		T	*P. haem* A (5)
	BR91-018	N	none
		T	*P. haem* A (UT)
53	BR91-017	N	*P. haem* T (3,4,10)
		T	*Proteus* overgrowth
	BR91-018	N	*P. haem* T (3,4,10)
		T	*P. haem* T (3,4,10)
55	BR91-018	TTW	*P. haem* T (3,4,10)
64	BR91-018	N	*P. haem* A (UT)
		T	*P. haem* A (UT)
			P. haem T (3,4,10)
72	BR91-017	N	*P. haem* A (5)
			P. haem T (3,4,10)
		T	*P. haem* A (5)
			P. haem T (3,4,10)
	BR91-018	N	*P. haem* A (5)
		T	*P. haem* T (3,4,10)
73	BR91-017	TTW	*P. haem* T (3,4,10)
143	BR91-019	N	*P. haem* 3
		T	*P. haem* 3
		TTW	none

[a] Samples: N = nasal, T = tonsillar, TTW = transtracheal wash.
[b] *Pasteurella haemolytica* biotypes A, T, and 3.
[c] *Pasteurella* serotypes in (), UT = untypable.

Table 3. *Pasteurella* spp. cultured from samples of bighorn ewes at time of capture and following caesarian-section delivery of lambs from 2 ewes and natural delivery by the third ewe, Caldwell, Idaho.

Sampling time	Ewe ID no.	Type of sample[a]	*Pasteurella* spp. and Serotypes isolated
At capture	BR91-003	N	None
		T	*P. haem* 3[b]
			P. haem T (3,4,10)
	BR91-008	N	None
		T	*P. haem* 3
			P. haem T (3,4,10)
	BR91-012	N	*P. haem* 3
		T	*P. haem* 3
			P. haem T (3,4,10)
2 days post caesarian	BR91-008	N	None
		T	*P. haem* 3
			P. haem T (3,4,10)
	BR91-012	N	None
		T	*P. haem* 3
			P. haem T (3,4,10)
			P. haem T (10)
		TTW	*P. haem* T (3,4,10)
			P. multocida
32 days post caesarian	BR91-008	N	None
		T	*P. haem* 3
			P. haem T (3,4,10)
	BR91-012	N	None
		T	*P. haem* T (3,4,10)
27 days post delivery	BR91-003	N	*P. haem* 3
		T	*P. haem* 3
			P. haem T (3,4,10)

[a] Samples: N = nasal, T = tonsillar, TTW = transtracheal wash.
[b] *P. haem* - *Pasteurella haemolytica* biotypes: T and 3; serotypes in ().
P. multocida - *Pasteurella multocida*: serotyping not conducted.

All nasal, tonsil, and transtracheal samples cultured negative for viruses. Low serum antibody titers were detected against several potential infectious agents (Table 1). However, the only titer considered significant was that against Anaplasma marginale.

DISCUSSION

Disease development in animals is dependent upon a number of factors including; the presence of infectious organisms in the environment, age and immunity of the host, genetic predisposition of an animal to specific diseases, nutrition, and stress (Biberstein 1981, Wiseman et al. 1978, Yates 1982). Most of our knowledge of respiratory disease in ruminants comes from studies of disease in cattle and domestic sheep. In these animals, virus infections of the respiratory tract are commonly associated with development of diseases caused, in part, by opportunistic bacterial pathogens (Carter 1973, Yates 1982). Pasteurella spp. are among the opportunistic bacterial pathogens which are commonly associated with viral infections which predispose their hosts to pneumonia. Infections with viruses or Mycoplasma spp. (Corstvet et al. 1973, Jensen et al. 1976) or infestations with parasites (Spraker 1979) which reduce the defense mechanisms of the lungs may contribute to respiratory pasteurellosis. Protostrongylus stilesi is a common parasite of bighorn sheep which is known to compromise the ability of the lungs to resist infection with opportunistic bacterial pathogens. These parasites may cross the placenta during the third trimester and infest the liver of lambs in utero. They subsequently migrate to the lungs after birth of the lambs and develop to sexual maturity at about 20 days. Larvae then develop and migrate up the bronchial tree causing granulomas in the lung. These events result in reduced resistance to infection with opportunistic bacterial pathogens (Spraker 1979).

Viruses were not isolated from samples collected from the caesarian-derived lambs prior to or at the onset of clinical pneumonia. However, P. haemolytica biotype T and P. stilesi were both present in lung lavage samples collected transtracheally. Both organisms appeared to have played a role in the pneumonic condition of the lambs. Antibiotic therapy improved the general appearance and responsiveness of the lambs but did not eliminate the persistent cough. However, following treatment with ivermectin, the cough response decreased, the lambs became more active, and antibiotic therapy was discontinued without subsequent recurrence of pneumonia.

Protostrongylus stilesi larvae were present in the lungs of the 2 caesarian-derived lambs monitored in this study. Due to age of the lambs at onset of disease and the mode of transmission of these parasites, it is evident that they would have been present in the lambs prior to birth. Thus, the lungworms were present prior to transmission of P. haemolytica from the ewes to their lambs initiating tissue damage resulting in increased susceptibility of the lambs to pneumonic pasteurellosis. Transplacental transmission of P. stilesi to lambs is common in free-ranging herds and may predispose lambs to pneumonia associated with particular strains of P. haemolytica to cause the "summer lamb mortality"

syndrome. Isolates of P. haemolytica are being evaluated to identify strains which are associated with this syndrome.

Pasteurella haemolytica is a diverse group of bacteria which appears to be ubiquitous in ruminants (Timoney et al. 1988). These opportunistic pathogens rarely initiate disease but are generally associated with disease only if other factors, such as viruses or lungworms, reduce the resistance of the host. Differentiation between P. haemolytica strains can be achieved by identification of genetic differences revealing "ribotypes" (Snipes et al. 1992) and DNA "fingerprints". The DNA fingerprinting procedure which provides highly discriminatory and reproducible information was subsequently conducted on P. haemolytica isolates from the lambs and their mothers to determine the source of the organisms associated with disease in the lambs (to be published).

Summer lamb mortality causing low recruitment rates in bighorn populations for 4-5 years following a pneumonic outbreak is devastating to the affected herds. Monitoring will continue in bighorn populations to identify additional factors which contribute to this syndrome in efforts to alter or remove those factors which result in disease.

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