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PREPARATION AND TESTING OF PASTEURELLA BACTERINS
ON CAPTIVE BIGHORN SHEEP

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Abstract

Experimental testing was done on two multi-valent Pasteurella bacterins: one a formalinized cell bacterin and the other an extracted cell bacterin. Two groups of captive-penned bighorn sheep were used for the tests. One group consisted of newborn full-blood and hybrid (F₂) bighorn sheep at Rachelwood Wildlife Research Preserve, Pennsylvania. This group received either antibiotics, domestic

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sheep anti-Pasteurella gamma globulins, and/or various doses of the formalinized cell bacterin after birth. The second group consisted of ten captive-penned lambs and yearling bighorn sheep at Sybille Big Game Research Unit, Wyoming. This second group received various doses of either the formalinized cell bacterin or the extracted cell bacterin. Indirect hemagglutination titers were determined as well as ability to survive natural infection following vaccination of the animals when held in pens and handled for the experimental studies. The formalinized cell bacterin and the extracted cell bacterin seem to protect newborn lambs and older bighorn sheep, respectively, from Pasteurellosis when given in multi-doses.

Introduction

Information is needed on Rocky Mountain bighorn sheep such as the physical conditions of individuals, biochemical normals, normal physiological parameters, nutrient requirements, breeding age, gestation period, multiple birth frequency and others. Bighorns must be held in captivity to gather this information. Bighorns are easily injured and readily contract pneumonia while in captivity. Post (1971) related the problems of respiratory diseases in bighorns and described the large variety of causes of pneumonia in bighorn sheep.

The "pneumonia complex" in bighorns is difficult to attribute to one cause. It may be a combination of effects. Problems with feed, crowding, handling, and stress of captivity as well as the microbial agents may be contributors to pneumonia. One organism which is reported most often as the primary cause of death in Rocky Mountain bighorns is Pasteurella. The organisms are found as normal flora in nasal passages (Post, 1962). Recent diagnosis of Pasteurellosis under semi-captive conditions have been reported in bighorns in Utah (Follis et al, 1971) and Wyoming (Thorne, 1971a).

The purpose of the research reported in this paper has been to prepare and test two Pasteurella bacterins for immunological response, ability to reduce the pneumonia caused by natural infection, and to aid in survival of bighorn sheep in captive conditions. Experimental animals were subjected to various stress conditions such as capture, handling, and alteration of feeding as well as close confinement. These are factors which must be overcome in order to hold them in confinement for any purpose. Immunological response was measured in terms of indirect hemagglutination titers.

Material and Methods

Bacterial Cultures - Six Pasteurella sp. isolated from nasal swabs and tissues of bighorn sheep were described previously by Nash (1971).

Bacterins - Formalinized multivalent cell bacterin (Bacterin A) was prepared with stock Pasteurella sp. using techniques given by Nash (1971). Two methods of antigen suspension were used. One suspension was in normal saline the other in mineral oil-lanolin (4:1) adjuvant. The adjuvant was added 1:1 to the saline cell suspensions and mixed to a final concentration of 5 mg dry wt bacterial cells/3 ml dose.

An extracted multivalent cell bacterin (Bacterin C) was prepared using a modified method of Herzberg et al (1972). It consisted of the residue of the living cells of the stock *Pasteurella* isolates after extraction with 2% deoxycholate in 0.2M tris, hydroxymethyl, amino methane buffer at pH 8.0 and 37 C. Suspensions were made in oil adjuvant similar to Bacterin A at 5 mg dry wt bacterial residue/3 ml dose.

Antiserum Production-Anti *Pasteurella* serum was prepared in domestic sheep using a 3 ml dose series of subcutaneously injected, saline suspended Bacterin A. Blood was taken from the animals six weeks after the last injection. The Gamma globulin fraction was concentrated using precipitation to a final indirect hemagglutination titer of 1:1,024.

Experimental Animals - Experiment No. 1 consisted of six newborn F₂ hybrid bighorn-mouflon lambs and two pure bred bighorn lambs from captive ewes at Rachelwood Wildlife Preserve, New Florence, Pennsylvania. All animals were held in enclosed pens on their mothers from birth.

Experiment No. 2 consisted of ten bighorn sheep captured near Dubois, Wyoming. Five animals (No's. 217, 218, 220, 223 and 224) were live trapped (Helms, 1970). The other five (No's. 215, 216, 219, 221, and 222) were captured with M-99 (Thorne, 1971b). All animals were transported by truck to the Sybille Wildlife Research Unit, Wheatland, Wyoming, confined to pens, and fed hay and protein supplements.

Treatment and Immunization - Experiment No. 1: The newborn lambs were given various treatments after birth. Each received either streptomycin (250 mg), various doses of antiserum, and either 2 or 3 doses of Bacterin A in saline suspension.

Experiment No. 2: Blood samples were taken from the jugular vein and each animal was vaccinated at the site of capture. Live trapped animals received Bacterin A in saline-oil adjuvant. Animals captured with M-99 received Bacterin C in saline-oil adjuvant. Three milliliter doses were given first intra-muscular in the right gluteus muscle. Additional doses were given either subcutaneously under the front legs or I.M. in the gluteus muscles. Two animals were held as experimental controls and received no bacterins, only the adjuvants. Blood samples and rectal temperatures were taken thereafter every two to four weeks.

Ten milliliters of blood were taken from the right jugular vein at each bleeding time, the serum was collected and *Pasteurella* antibody titers were determined. Indirect hemagglutination titers were determined using formalinized sheep red blood cells and the 0.025 ml microtiter techniques previously described by Nash (1971).

Results

The newborn bighorn and bighorn-mouflon hybrid lambs which received the complete series of antibiotics when needed, antiserum, and three doses of Bacterin A survived the longest (Table 1). One animal survived for almost six months, another survives today (over one year).

The dead animals generally showed chronic pneumonia. Mycoplasma were isolated from animals 6-B and 9-C (bighorn lambs). No causative microorganisms could be isolated from the bighorn-mouflon hybrids at death. No Pasteurella sp. were isolated from any of the animals in Experiment No. 1.

Results obtained from the ten older bighorn sheep captured from the wild are given in Table 2. Body temperatures were not taken at capture to minimize stress. However, the average rectal body temperature at capture of wild bighorns was taken as 101.9° F and used as a reference point (Franzman et al, 1971). Note that Groups 2 and 4 showed a rapid increase in body temperature after capture and dropped after four weeks in captivity. The experimental control group showed a rapid increase in body temperature for the first 4 weeks.

The average indirect hemagglutination titers for the various groups of bighorn sheep are shown by Graph 2. Groups 2 and 4 show the highest average titers over the longest period of time. The experimental controls show little or no titers to the selected Pasteurella sp.

All vaccinated animals showed some degree of pneumonia after five weeks of captivity. Necropsy results showed general consolidation of the apical, cardiac and anterior diaphragmatic lobes of the lungs. There was usually no purulent exudate in these animals. Group 4 lived the longest and showed no Pasteurella sp. or apparent causative agent.

The experimental control animals showed pneumonia with purulent abscesses throughout the lungs as well as collections of edematous fluid in the thoracic cavity. There were numerous adhesions and almost pure cultures of Pasteurella sp. from the lungs and heart blood.

Discussion

Limited data have been published on the treatment and raising of newborn lambs in captivity. Howe (1966) was able to hold three young bighorn lambs taken from their mothers out of the pasture in isolation and free from pneumonia for at least 40 days after birth. These animals all died of pneumonia three to four weeks after being released into pens. Data from Table 1 indicates the Pasteurella bacterin is of some value in the survival of newborns. Time seems to be an important factor as well as the health and physical state of the animal at birth. Those animals in this study which lived to be at least 48 days old and received at least two doses of bacterin lived about twice as long as those receiving no bacterin. Those that received three doses have lived six months to at least one year with one exception, bighorn lamb 6-B received no bacterin, antiserum, or antibiotics, yet survived for 151 days. No Pasteurella sp. were isolated from any of the lambs which died.

Results shown by Experiment No. 1 indicate that antibiotics, antiserum, and bacterin should be given at birth followed by daily doses of antibiotics for at least 7 days. The antiserum is short lived and gives only immediate passive immunity. It takes from 7 to 14 days to stimulate a high antibody response to the bacterins. Additional doses of bacterin should be given at 21 day intervals for two more doses and then repeated every six months if the animals are confined in pens. The three week period is used so that the animals are handled less and placed under as little stress as possible.

Table 1

RECORD OF TREATMENTS AND SURVIVAL OF NEWBORN BIGHORN AND BIGHORN
HYBRID LAMBS
(Experiment No. 1)

NUMBER	SEX	BREED	BORN	DIED	SURVIVAL Days*	Treatments Received			BACTERIN Days*
						ANTIBIOTIC Days*	ANTISERUM ml Days*		
6-B	F	Bh	5/31/71	10/29/71	151	-	-	-	-
9-C	F	Bh	4/13/71	10/29/71	198	-	0.8	21	49;70;91
H71-2	M	F ₂ Hy	4/27/71	5/22/71	25	7	0.4	7	-
H71-3	M	F ₂ Hy	4/25/71	5/31/71	36	9	0.6	9	-
H71-5	F	F ₂ HY	4/12/71	7/6/71	86	-	-	-	48;70**
H71-6	M	F ₂ Hy	4/13/71	7/20/71	98	21	0.7	21	47;69
H71-7	M	F ₂ Hy	4/14/71	7/21/71	98	20	0.8	20	46;68
F27-C	M	F ₂ Hy	4/7/71	†	365+	13	0.6	13	48;69;90

*Days following birth.

Bh= full-Blood Bighorn.

F₂Hy= F₂ Bighorn-Mouflon Cross.

**= Age of animal at time of vaccinations. The number of Recorded Ages indicates the number of 3 ml S.Q. inoculations of Bacterin A in a saline suspension.

-- No treatment of the given substance was given to that animal.

†= This animal is still living.

TABLE 2

RECORD OF CAPTURE, HANDLING, BLEEDING, TEMPERATURES, AND VACCINATIONS DONE ON BIGHORN SHEEP
HELD IN CAPTIVITY IN CONFINED PENS
(EXPERIMENT NO. 2)

GROUP NUMBER	SEX	WT. LBS.	AGE	BACTERIN	Dates of Various Treatments				Pasteurella Isolated			
					12/20/71	1/6/72	1/20/72	2/10/72		3/9/72		
1	218	M	65	L	A	CBV	HBVT	HBVT	-	-	-	+
1	220	F	100+	1 yr	A	CBV	HBVT	HBVT	-	-	-	+
2	217	M	80	L	A	CBV	HBVT	HBVT	-	-	-	+
2	224	F	106.5	2 yr	A	CBV	HBVT	HBVT	-	-	-	+
3	221	F	122.5	1 yr	C	CBV	HBVT	HBVT	-	-	-	-
3	219	F	96	1 yr	C	CBV	HBVT	HBVT	-	-	-	-
4	215	F	100	1 yr	C	CBV	HBVT	HBVT	HBV	HBVT	HBVT	-
4	216	F	102	1 yr	C	CBV	HBVT	HBVT	HBV	HBVT	HBVT	-
5	223	M	66	L	Ex.Con.	CBI	HBVT	HBVT	-	-	-	+
5	222	F	100	1 yr	Ex.Con.	CBI	HBVT	HBVT	HB	HB	HB	+

L= Less than one year old.

C= Date captured is one of three dates 12/20/71, 12/21/71, and 12/22/71 and are given as one date for ease of recording data.

B= Blood samples taken on this date

H= Animal handled on this date.

V= Animal vaccinated on this date.

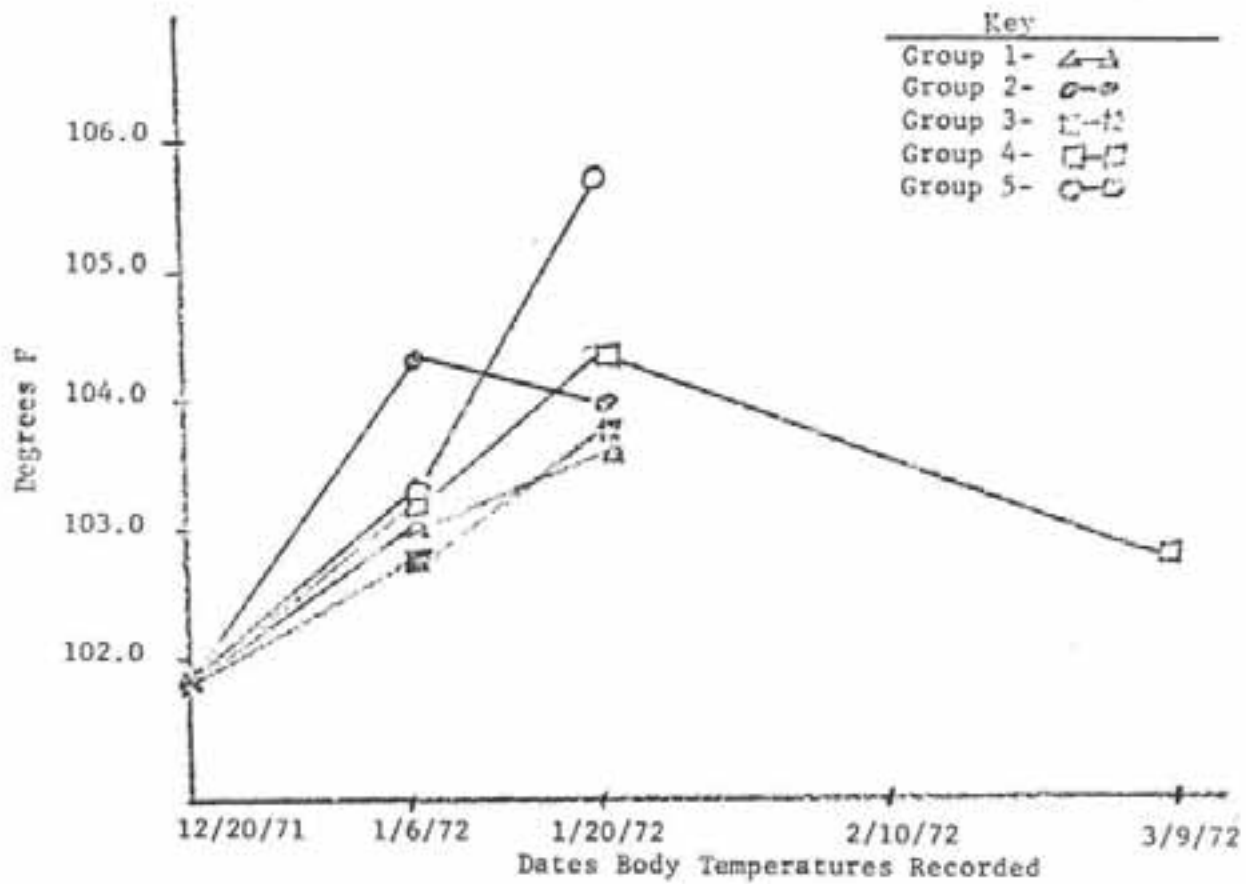
I= Experimental control animal was given a placebo.

T= Temperatures taken on this date.

Ex.Con.= Experimental Control animal.

Graph 1

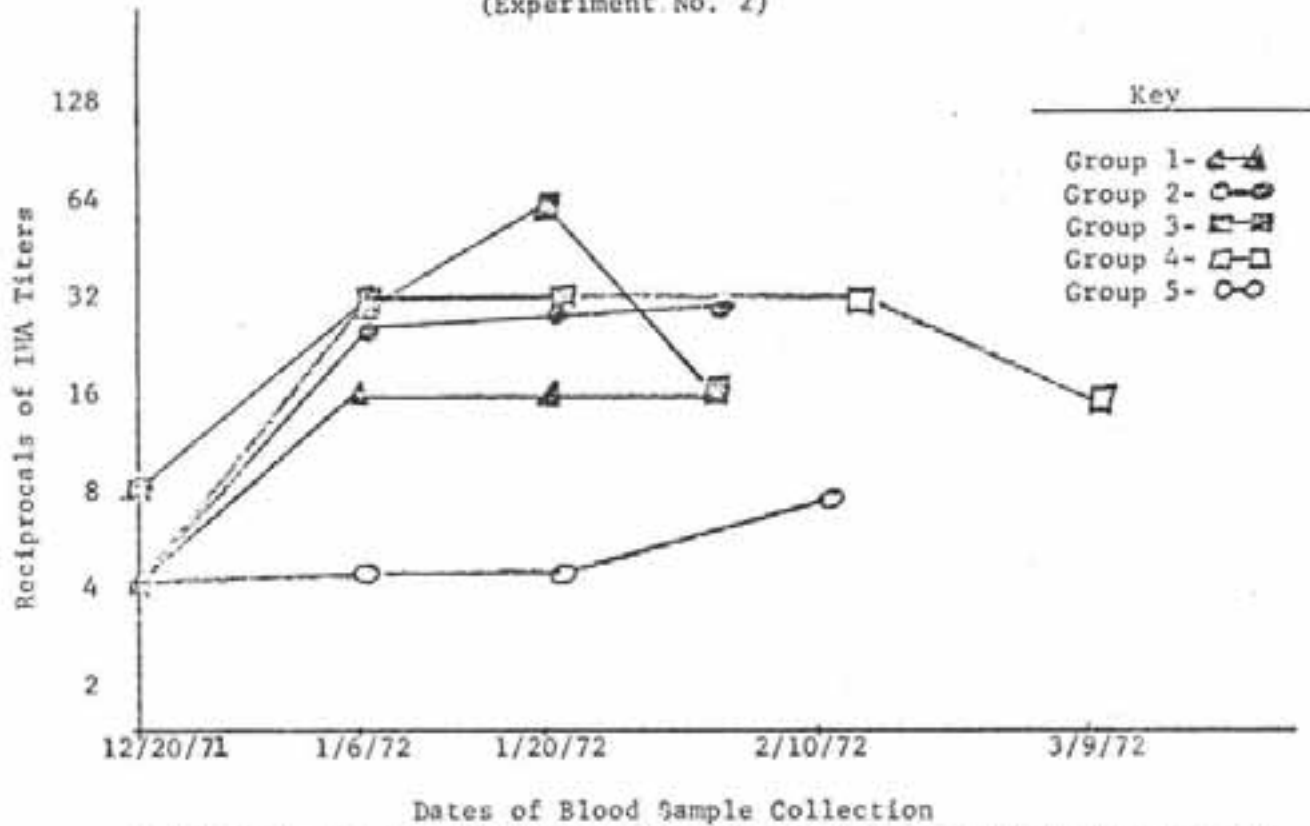
RECTAL BODY TEMPERATURES OF BIGHORN SHEEP
(Experiment No. 2)



* Estimated as 101.9 F (Franzman et al, 1971).

Graph 2

INDIRECT HEMAGGLUTINATION TITERS* RECORDED ON DATES AFTER CAPTIVITY
(Experiment No. 2)



* Given as average IHA titers for the two animals in each group or the average for one animal if one animal died before this reading.

There is little available data on the holding of older bighorn sheep taken direct from the wild and held in enclosed pens. Bingham (1962) attempted an experiment to test a Pasteurella bacterin on six bighorn ewes. The animals were confined to pens during the experiment. The experiment had to be discontinued when four animals died. Two animals showed pneumonia with isolation of a Pasteurella sp. The other two died from enterotoxemia. Woolf (1971) reported an influence of lambing and morbidity on body weights of 8 captive bighorn sheep. Franzman et al (1971) held six bighorns for at least two weeks in confinement.

There is no need for a direct challenge of living bacteria to test immunity produced with a bacterin against pneumonia in bighorn sheep when animals are confined to pens. These animals are probably exposed to natural infections while under intense stress conditions of capture, handling, bleeding, vaccination and other treatments. Table 2 shows that the animals in Group 4 were subjected to all of these factors. The use of Bacterin C seemed to lower the intensity of the pneumonic symptoms. This could have been because Pasteurella sp. were not present prior to the time of death to intensify pneumonic symptoms.

Franzman et al (1971) and Thorne (1971b) have shown that the physiological values of bighorns increase quite rapidly when captured. Excitability due to capture, handling, bleeding, taking body temperatures and vaccinations all add to the total stress placed on a wild animal. Significant changes occur to bighorn sheep under these conditions. The animals in Group 4 remained alive and in excellent health with no signs of pneumonia for eighty-two days. Graph 2 shows that the immunological response from the three doses of Bacterin C was the greatest and the titer remained higher for a longer time. The titer drop seen after 62 days may be only a variation in interpretation of tests and not a true reduction in circulating antibody. The continued vaccination may possibly have paralyzed the immune response of the animals.

Graph 1 shows similar results of the other captive bighorn sheep by a rise in body temperature from time of capture and for the first two weeks of captivity. Franzman et al (1971) showed an increase of almost 3° F after holding the animals in pens for two weeks. The body temperature rise in Groups 2 and 4 may have been caused by the handling procedures received by the animals. Group 5, the experimental controls, showed a rapid increase in body temperature which did not drop. This may indicate the chronic pneumonic condition found at death. The use of experimental Pasteurella bacterins seemed to reduce body temperatures over a period of time. Follis et al (1971) showed similar results when a Pasteurella bacterin was used on semi-captive bighorns.

Dalton et al (1971) pointed out that similar problems occur in direct transplants of bighorn sheep without confinement. Stress of capture and handling may weaken the animals and they contract pneumonia. Treatment of these animals should follow the same procedures as for confined animals. Animals should be immobilized with a drug such as M-99 (Franzman et al, 1971, and Thorne, 1971b), to reduce excitability at capture. The authors recommend a broad spectrum antibiotic be given immediately as should Pasteurella sp. bacterin speci-

fically produced from strains of Pasteurella isolated from bighorns. Antibiotic treatment should continue for at least 7 days if the animals are to be held in captivity. Animals should be revaccinated from two to three more times at 2 week intervals. Multi-site vaccination should be used to obtain maximum stimulation of the lymphatic system and to reduce tissue damage in the animals. This procedure may reduce the losses from pneumonia. The same procedure should be followed on direct transplants except that multi-site single vaccination (rump and shoulder) should be used.

The research reported here does not offer an absolute control for pneumonia in captive bighorn sheep. There is an indication, however, that the bacterin may be useful in controlling invasion of Pasteurella sp. in captive animals.

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