VACCINATION OF AMERICAN BISON AGAINST *Pasteurella multocida* SEROTYPE 2 INFECTION (HEMORRHAGIC SEPTICEMIA)

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Abstract: Following an epizootic of septicemic pasteurellosis in a herd of American bison in 1965, calves have been vaccinated each fall. There have been no further signs of the disease. Results of bacteriological and serological studies on this herd are reported. *Pasteurella multocida* and *Pasteurella haemolytica* were not isolated from nasal cavities. Antibodies against *P. multocida* serotypes 1 (pronghorn strain), 2 (vaccine strain), and 4 (black bear strain) were detected in serum samples from some bison. No antibodies were detected against serotype 3, a bovine strain originally isolated from the lung of a calf. All serum samples contained antibodies against parainfluenza-3 virus.

INTRODUCTION

An epizootic of septicemic pasteurellosis (hemorrhagic septicemia, HS) caused by *Pasteurella multocida* serotype 2 (Roberts type I, Carters type B) occurred in a herd of American bison *(Bison bison)* in 1965 at the National Bison Range, Moiese, Montana.[10] Young animals were most severely affected. Since 1966, calves have been vaccinated with a formalin-inactivated vaccine prepared at the National Animal Disease Laboratory (NADL), and no further signs of HS have been observed in the herd. To further assess the vaccination program and to determine if microbial agents associated with "shipping fever" (pneumonic pasteurellosis) were present in the herd, nasal swabs and blood samples were taken for bacteriological and serological examination.

No extensive review of the literature on HS is included in this report as it is comprehensively reviewed by R. V. S. Bain.[2] Hemorrhagic septicemia was defined at the 2nd International Meeting on HS[4] as a specific disease caused by a specific serotype of *P. multocida* which may have antigenic variations. It is an infectious, septicemic disease of cattle, bison, water buffalo and to a lesser extent, of various other animals such as camels and swine. The disease seldom occurs in the United States, having only been confirmed in American bison in 1912,[13] 1922,[9] and 1967,[10] and in young dairy cattle in 1969.[12] In several countries the disease is quite prevalent and is usually controlled with a water-in-oil emulsified vaccine similar to the one used on the bison.[2] Isolants of *P. multocida* from cases of HS have been given various serotype designations by different people using various serologic methods,[10] but it was primarily the observations of workers in Asia that established that a specific serotype causes HS.[1,3,11]

According to Dr. Cora Rust Owen, Dept. of Health, Education and Welfare, Rocky Mountain Laboratory, Hamilton, Montana, signs, consisting of stiff and swollen limb joints, were observed in some calves on the bison range during the years 1963-64 (personal communication). In 1965,[10] Dr. Owen isolated *P. multocida* strain P-1459 from a limb joint of a bison. Since 1966, strain P-1459 has been used to prepare an inactivated vaccine for the bison calves.

MATERIALS AND METHODS

During the 1972 fall roundup (Figure 1), the bison were examined for general physical condition and all calves were branded and vaccinated for HS using a vaccine prepared as previously described.[10] Young heifers were also vaccinated for brucellosis. To prevent overgrazing, surplus animals were removed from the herd and sold in order to maintain about 400 bison on the range. From approximately 75 animals that were cut from the herd in 1972, 41 blood samples and 55 nasal swabs were collected. Each animal was run into a squeeze-chute where 8 ml of blood was taken from the jugular vein. Nasal swabs were collected by inserting a serile, cotton-tipped, 6-inch wooden applicator into the right nasal cavity. They were streaked on dextrose starch agar (BBL) plates and then twirled in screw-capped vials containing 6 ml of tryptose broth (Difco). The broth and plates were refrigerated at approximately 4 C and transported to the NADL where the broth was incubated 4 hours at 37 C and then streaked on dextrose starch agar plates and blood agar base (Difco) plates containing 5% difibrinated bovine blood. All plates were incubated 20 hours at 37 C and examined for colonies of *P. multocida* and *P. haemolytica* using a stereomicroscope with obliquely transmitted light.[7]

The gel diffusion precipitin test[9] was utilized to determine if antibodies were present in the serum against the following strains of *P. multocia:* the vaccine strain P-1459 (serotype 2),[10] a bovine strain P-1062 (serotype 3),[10] an isolant P-1495 (serotype 1) from a pronghorn antelope *(Antilocapra americana)* and an isolant P-1510 (serotype 4) from a black bear *(Euarctos americanus).* Isolant P-1495 is one of two isolants from the livers of two pronghorns that were found dead on the National Bison Range, and P-1510 is from the mouth of a black bear in the Bitterroot Valley of Montana (no history). The cultures were iso-

FIGURE 1. Cutting bison (buffalo) from the main herd and running them into a corral for inspection.

lated in 1966 by Dr. Owen and serotyped at the NADL. The pronghorn isolants were the same serotype that we have observed in cultures from waterfowl in California, Colorado and Utah.

The mouse protection test was conducted using 16- to 20-g Carworth white female mice (B. D. Laboratory Animals, Portage, Michigan); 0.2 ml of each serum sample was inoculated intraperitoneally (ip) into each of five mice. Twenty-four hours later, approximately 1100 organisms (1100 lethal doses) of strain P-1459 were challenge-inoculated ip into the 5 test mice and 20 control mice. To evaluate the degree of passive immunity or Protection Index (PI) induced by the serum, each mouse was allotted 2 points for each day of survival. Thus, a serum sample scored from 0 to 100 using the following PI formula:

$$\frac{100}{N_d \cdot N_a} \cdot \sum_{i=1}^{N_a} X_i = \text{Protection Index}$$

N_d = No. of days of test (should remain constant at 10)

N_a = No. of mice

X_i = No. of days the i^{th} mouse survived

For example, if all 5 mice died within 24 hours after challenge exposure, the PI was 0; but if all mice survived for 10 days, the PI was 100.

The hemagglutination-inhibition test was used to determine if antibodies were present against parainfluenza-3 virus,[5] an agent frequently associated with pneumonic pasteurellosis.

RESULTS

Pasteurella multocida and *P. haemolytica* were not isolated from the nasal cavities. Results of the serological test with the vaccine strain of *P. multocida* P-1459 and parainfluenza-3 virus are presented in Table 1. Results of the gel precipitin test with the bovine isolant P-1062 isolant were negative, with the bear isolant P-1510, two serum samples were positive, and with the pronghorn isolant P-1495, 15 serum samples were positive. Sixty-six percent of the serum samples had a PI of 20 or greater (20 of 20 control mice died in less than 20 hours) and produced a precipitin line with the vaccine strain P-1459. Six of 26 (23%) serum samples with a PI of 20 or greater did not produce precipitin lines, and 6 of 15 (40%) with a PI of less than 20 did produce precipitin lines. Antibodies against parainfluenza-3 virus were present in all serum samples tested (Table 1).

DISCUSSION

It is not known what part the vaccine has played in preventing HS in the bison herd, but because no further signs of HS have been observed it would seem that the use of the vaccine in the bison calves during the fall roundup is warranted.

Bovine shipping fever (respiratory form of pasteurellosis), which is thought to be caused by parainfluenza-3 virus in association with secondary invaders, *P. haemolytica* and *P. multocida* other than serotype 2, has never been reported in the bison. However, since *P. multocida* serotype 1 was isolated from two dead pronghorn antelope from the National Bison Range, we tested for antibodies against serotype 1 and also 3 and 4 which are the most prevalent types that have been associated with pneumonic pasteurellosis of cattle.

Although *P. multocida* serotype 1 was isolated from the livers of the two dead pronghorns, the cause of death is not known. They may have had a respiratory infection which developed into a septicemia just before death as sometimes occurs in bovine calves. The only known relationship between the postmortem findings of the bison and the pronghorns was that *P. multocida* was isolated from tissue of both species. To isolate *P. multocida* from the bison and pronghorns, infected tissues were inoculated into mice that subsequently died of acute septicemia, and *P. mutocida* was isolated in pure cultures from the mice. Under

TABLE 1. Serological results of mouse protection test (Protection Index, PI) and gel precipitin (gel P.) tests using **P. multocida** culture P-1459 and hemagglutination-inhibition (HI) test of bison serums samples against parainfluenza-3 virus.

Bison age, years	Serological test			Bison age, years	Serological test		
	PI	Gel. P.	HI		PI	Gel. P.	HI
1	0*	—**	32†	4	46	—	256
1	0	—	32	3	50	—	
2	0	+	64	1	54	+	16
1	2	—	8	1	64	+	64
1	2	+	32	2	64	+	64
1	2	—	8	1	66	+	32
2	2	—	32	3	68	+	32
1	4	+	32	1	74	+	16
2	4	—	128	1	80	+	32
1	8	+	64	1	82	+	
1	8	+	32	1	82	+	16
1	14	—	64	3	82	+	32
1	14	—	16	17	82	—	256
2	16	+	64	1	84	+	32
6	18	—	64	5	84	+	64
1	22	—		1	94	+	64
1	22	+	128	1	100	+	16
1	28	+	16	1	100	+	16
5	28	—	128	1	100	+	64
1	40	—	16	1	100	+	128
				2	100	+	32

* Numerical score based on 5 mice; each mouse was given 2 points for each day of survival, with a possible score of 100 if all survived 10 days.

** — = No precipitin line; + = precipitin line observed.

† Reciprocal of highest serum dilution causing complete inhibition of hemagglutination.

these circumstances, in the days before serotyping, one probably would have assumed that both the bison and pronghorns died of HS that was caused by the same strain of *P. multocida*. Such events would account for some of the confusion associated with the early studies of pasteurellosis.

Bain reported that the mouse protective test is satisfactory for measuring immunity in either vaccinated or naturally immune animals.[1] Survival of any test mice, provided that all controls die, indicates the presence of protective antibodies. He suggested using between 0.1 and 1.0 ml of serum per mouse. In this study, 0.2 ml of serum was used. If more or less serum had been used, the results may have been quantitatively different, but the conclusion that the bison had protective antibodies against the vaccine strain would have probably been the same.

There was not always correlation between the results of the passive immunity test in mice and the gel diffusion precipitin test, in that some serum samples which induced passive immunity in mice did not produce a precipitin line, which is similar to previous observations.[8] We have recently observed (unpublished) that serum from some vaccinated turkeys which are immune to fowl cholera will passively immunize poults, but will not produce a precipitin line unless the serum is concentrated.

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