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A SEROLOGICAL SURVEY FOR SELECTED VIRAL INFECTIONS OF ROCKY MOUNTAIN BIGHORN SHEEP

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Abstract: Serum samples were collected from 29 Rocky Mountain bighorn sheep *(Ovis canadensis)* in their natural habitats in Colorado and Wyoming. Sera were tested for the presence of antibodies to six viruses. Hemagglutination inhibition tests indicated 18 sheep had antibodies to PI3, with 9 having titers greater than 1:8 and 24 sheep had antibodies to bovine parvovirus 1, with 24 having titers greater than 1:8. Serum neutralization tests showed one sheep had antibodies to the viruses of bluetongue and bovine viral diarrhea while no animals had titers to infectious bovine rhinotracheitis. Complement fixation tests revealed 13 sheep had antibodies to the group specific CF antigen of the adenoviruses. Only one animal possessed the group specific precipitating antibody.

INTRODUCTION

Viruses as causes of disease in wild bighorn sheep have not been investigated extensively. Parainfluenza-3 (PI_3),[10] bluetongue[11] and contagious ecthyma[1] viruses have been recovered from bighorn sheep, but neither their prevalence nor their importance have been fully elucidated. It was because of this paucity of information that a serological survey seemed appropriate. Viral agents included in this survey were selected because of their previous reports in Rocky Mountain bighorn sheep or other forms of wildlife; or because of the likelihood of their causing disease in wildlife. It was hoped that this survey would give an indication of which viral diseases are most prevalent in Rocky Mountain bighorn sheep in their natural habitat.

MATERIALS AND METHODS

Serum: Blood was collected from 29 individuals by heart or venous puncture immediately after trapping or shooting. Serum was removed as soon as possible, inactivated at 56 C for 30 minutes and stored at —20 C for future use.

Animals ranged in age from 1 month to 2½ years of age and were collected from various locations throughout Colorado and Wyoming (Table 1).

Serological Tests: Hemagglutination inhibition (HI) titers were determined by the microtiter method for PI3[10] and bovine parvovirus 1.[12] Bovine erythrocytes were used with PI3 and guinea pig erythrocytes were used with bovine parvovirus 1. Eight hemagglutinating units of virus were used in each case.

Tissue culture virus neutralization tests were used for detection of antibodies to infectious bovine rhinotracheitis[9] (IBR), bluetongue[7] (BT) (BT 8 strain) and bovine viral diarrhea[4] (BVD) (NADL strain) viruses. One hundred $TCID_{50}$'s of virus were used in each instance. Madin-Darby bovine kidney (MDBK) cells were used for IBR virus, primary embryonic bovine kidney (BK) cells for BT virus and primary embryonic spleen (BES) cells for BVD virus neutralization tests. All cells were propagated in minimum essential medium (MEM) contain-

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TABLE 1. Viral Antibodies in Sera of Rocky Mountain Bighorn Sheep

Sheep No.	PI_3[1]	IBR[2]	BVD[2]	BT[2]	Parvo[1]	Adeno CF[10]	Adeno ID	Age in Mos.	Location of Capture
1	2	0	0	0	128	0		1	TM[3]
2	8	0	0	0	16	NA	NA	2-3	TM
3	8	0	NA	NA	8	NA	NA	3-4	BP[4]
4	2	0	0	0	64	NA	NA	4-5	PP[5]
5	64	0	0	0	32	0	—	4-5	PP
6	8	0	0	0	128	NA	NA	4-5	PP
7	4	0	0	0	128	NA	NA	4-5	PP
8	0	0	NA	0	0	NA	NA	4-5	PP
9	8	0	64	64	512	4	—	1-2	PP
10	16	0	0	0	16	4	—	4-5	PP
11	0	0	0	0	64	2	—	4-5	PP
12	4	0	0	0	64	8	—	4-5	PP
13	4	0	0	0	32	8	—	4-5	PP
14	0	0	0	0	16	4	—	4-5	PP
15	0	0	0	0	0	4	—	4-5	TM
16	4	0	0	0	0	4	—	5-6	BP
17	4	0	0	0	4	8	—	30	ME[6]
18	32	0	0	0	64	2	—	12	WM[7]
19	16	0	0	0	32	0	—	12	WM
20	0	0	0	0	64	4	—	7	WM
21	0	0	0	0	16	0	—	8	WM
22	8	0	0	0	64	0	—	12	WM
23	0	0	0	0	16	16	—	12	WM
24	0	0	0	0	32	4	—	24	WM
25	0	0	0	0	32	0	—	24	WM
26	0	0	0	0	128	0	—	7	WM
27	4	0	0	0	4	0	—	1	WM
28	4	0	0	0	32	AC	+	?	SC[8]
29	0	0	0	0	8	NA	NA	?	RW[9]
% Pos.	62.1	0	3.7	3.6	89.7	62%			

Legend:

1. Titer expressed as reciprocals of highest serum dilution with complete hemagglutination inhibition.
2. Titer expressed as reciprocal or highest serum dilution with complete neutralization of 100 $TCID_{50}$'s of virus.
3. Trickle Mountain, Colorado.
4. Buffalo Peak, Colorado.
5. Pike's Peak, Colorado.
6. Mt. Evans, Colorado.
7. Whiskey Mountain, Wyoming.
8. Sandcreek, Wyoming.
9. Rachelwood, Wyoming.
10. Titer expressed as reciprocal of highest dilution with 50% complement fixation.

NA = not available for test.

AC = anticomplimentary serum.

ing 10% fetal calf serum (FCS)[8] and maintained with 2% FCS. Primary cells were initiated according to the method of Madin.[8]

The complement fixation test was performed as described by Darbyshire and Pereira[2] using the microtiter method. An adenoviral group specific CF antigen was purchased from Microbiological Associates. Immunodiffusion tests were performed as described by Yiu-Coggrave[14] using Cordis (Cordis Labs., Miami, Florida) IDF-II cells. The CF antigen was also used for the ID antigen, and the cells were incubated at 4 C for 72 hours.

RESULTS

Table 1 contains a summary of the results obtained in this serological survey. Antibodies to PI3 virus were detected in 18 (62%) of the 29 animals surveyed. In 9 of the sheep HI titers were 1:8 or higher. None of the animals had antibodies to IBR virus. An individual sheep was shown to have antibodies to BVD and BT viruses while the remainder of the animals tested were free of both viral antibodies. Bovine parvovirus 1 antibodies were demonstrated in 26 (90%) of the 29 sheep tested.

Adenovirus groups specific CF antibodies were demonstrated in 13 (62%) of 21 animals surveyed. Four of these 13 animals had titers of 18 or greater. Of 22 animals surveyed by ID for group specific precipitin antibodies, only one animal (no. 28) was a positive reactor; this animal's serum was anticomplimentary, so application of the ID test was not accomplished.

DISCUSSION

The results seem to indicate that BVD and BT viruses are not common infections among bighorn sheep of Colorado and Wyoming. Only one lamb (no. 9) had antibodies to the two viruses. This particular lamb was captured alive and we were able to follow its antibody titers over a period of time. It was approximately 1 month of age at the time of capture and serum was collected biweekly for an additional 3 months. Antibody titers to all viruses declined at a rate consistent with the decay of passive antibodies. The lamb, at the time of this writing, is healthy and has only negligible levels of viral antibodies. Presumably the initial titers obtained were passive antibodies obtained from colostrum and did not indicate an active infection. Bovine viral diarrhea virus has not been reported in Rocky Mountain bighorn sheep so our results of only one case are not unexpected. However, BT has been incriminated as being partly responsible for the disappearance of the desert bighorn sheep in Texas.[11] Our results compare favorably with those of Trainer and Jochim,[13] whose serological survey for BT antibodies revealed no positives in Wyoming and Montana while 53% of the bighorn sheep in New Mexico had titers. Perhaps BT is a problem of desert bighorn sheep in the southwestern parts of the United States, but does not occur in the Rocky Mountain area.

None of the animals had antibody titers to IBR which is in agreement with a previous survey.[5]

Parainfluenza-3 virus has been incriminated as a pathogen in bighorn sheep by serological evidence[5] and by virus isolation.[10] This study, which revealed PI3 antibody titers in 62% of the sheep, indicates that the virus is widespread and could be an important pathogen.

The results obtained with bovine parvovirus 1 are the most puzzling. Ninety percent of the animals possessed antibody titers. This is surprising since a previous survey[12] showed that domestic sheep did not have antibody titers to bovine parvovirus 1. The pathogenic potential of bovine parvovirus 1 has not been fully elucidated in cattle and it is impossible to make any statement as to its importance in Rocky Mountain bighorn sheep.

Adenoviruses were suspected of infecting cattle, horses, and sheep because of serologic evidence.[2] Subsequently, adenoviruses were recovered from these animals, and some of these animals had respiratory infections associated with the recovered adenovirus.[6,14] Adenoviruses have not previously been incriminated

in the bighorn sheep respiratory syndrome, but the existence of group specific adenoviral CF antibodies in 62% of the individuals examined suggests that these viruses could be involved.

From the findings of this investigation we may surmise that PI_3 and adenoviruses are commonly encountered by bighorn sheep and may be important agents of disease, based on their role in other species of animals. Either virus or both could possibly be involved in the important pneumonia complex of bighorn sheep, since they are frequently incriminated in respiratory disease. The incidence of bovine parvovirus 1 antibodies was extremely high, indicating widespread contact with the virus, but its importance is unknown. Bluetongue, IBR, and BVD viruses were insignificant factors based on the number of animals possessing antibody titers to the agents.

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