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Infection of Bighorn Sheep (*Ovis canadensis*) with *Myxovirus parainfluenza-3* and Other Respiratory Viruses. Results of Serologic Tests and Culture of Nasal Swabs and Lung Tissue

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ABSTRACT

Hemagglutination-inhibition (HI) tests of serum samples from 51 bighorn sheep (*Ovis canadensis*) and 4 bighorn-domestic hybrid sheep from Wyoming and Montana revealed bovine myxovirus parainfluenza-3 antibody titers of 1:20 or greater in 6 animals. The serums of two bighorns with pneumonia were negative for neutralizing antibodies against IBR virus and the serums of 9 animals failed to show significant titers against 2 strains of human Asian influenza virus. Tissue cultures of nasal swabs and lung tissue were negative for cytopathogenic effects and hemadsorption.

INTRODUCTION

Soon after the introduction of domestic livestock into the Rocky Mountain region herds of the once abundant bighorn sheep (*Ovis canadensis*) were reduced to a few scattered groups. Although ecological restrictions were important in this reduction, disease agents carried by the livestock and apparently introduced to the sheep took a heavy toll. An epizootic described as "scab" (1880-1900) was followed by outbreaks of pneumonia which have persisted until the present time (Baillie-Grohman, 1882; Buechner, 1960; Honess and Frost 1942; Marsh, 1938; Moser and Pillmore, 1954; Packard, 1946; Smith, 1956; and Wright, *et al.*, 1933).

The discovery of the lungworm *Protostrongylus stilesi* in bighorn sheep in 1927 led some investigators to implicate this parasite as a predisposing factor to a usually fatal bacterial infection (Marsh, 1938). However, the epizootiology and pathology of the verminous and bacterial diseases have not always supported this theory. Thus, the involvement of viral agents was considered.

The pneumonia was generally acute, running a short fatal course in lambs but occasionally became chronic in older animals that survived the acute stage. Captive animals were treated with intravenous or intramusular injections of exytetracycline, penicillin, streptomycin and tylosin which gave only

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temporary relief from clinical signs. Acute injections were characterized by fever, hyperpnea, anorexia, and depression. As the condition became chronic intermittent coughing developed and dyspnea occurred in the terminal stages. Recurrence of the disease after antibiotic treatment usually resulted in death of the animals. Preliminary studies on the role of viruses in the pneumonia complex in bighorn sheep have been completed and the results are reported.

MATERIALS AND METHODS

Serum samples from 55 bighorn sheep were tested for hemagglutination-inhibiting (HI) antibodies against bovine myxovirus parainfluenza-3 (SF-4 virus) with a method previously described (Woods, *et al.*, 1961). The source of the animals (Fig. 1) was as follows: 9 rams from Montana; 25 animals live-trapped from a wild herd in Wyoming; 17 captive Wyoming animals including 3 showing signs of pneumonia; 4 bighorn-domestic hybrid sheep. The Montana sheep were mature rams taken from Wild Horse Island in Flathead Lake and the National Bison Range near Moiese. The Wyoming animals were primarily mature ewes plus a few lambs and young rams. All were obtained from the Wind River Mountains, and some were being kept in captivity at the Sybille Experimental Unit when the blood samples were taken. Two of the pneumonic captive bighorns were also tested for infectious bovine rhinotracheitis (IBR) antibodies, and serums from the 9 Montana sheep were tested against 2 strains of human Asian influenza virus-A_2/Japan/305/57 and A_2/Japan/170/1962. The methods were described previously (Woods *et al.*, 1962).

Nasal swabs from 22 of the live-trapped Wyoming bighorns and 13 of the captive animals were cultured on bovine embryonic tissue for cytopathogenic agents. Swabs, received frozen in Hank's solution, were thawed and 0.2 cc inoculated into 2 tubes of primary bovine kidney tissue maintained in medium 199. The tubes were placed on a roller drum at 35 C for 7 days and then removed and examined for cytopathic effect (CPE). The fluid was then removed, the cell sheet washed twice with phosphate buffer and 0.2 ml. of 0.5% guinea pig red blood cells were added to each tube. The tubes were incubated in a stationary position at 4 C for 30 minutes and then observed microscopically for red cell hemadsorption.

Lung tissue from 15 animals was ground with a sterile mortar and pestle to a 10% suspension by weight with sterile sand and medium 199. Four tubes of bovine kidney cell monolayers were each inoculated with 0.2 ml. of this suspension. The evaluation of the results was described above.

Brain-heart infusion agar was also inoculated from the nasal swabs.

RESULTS

Only one of the Montana bighorns showed an HI titer greater than 1:10 against SF-4 antigen. This was a 7-year-old ram from the National Bison Range, which had a titer of 1:20. Titers against both strains of human Asian influenza viruses were less than 1:10. The only detectable HI titers against SF-4 virus in the Wyoming bighorns were in 3 wild live-trapped mature ewes (1:80, 1:80 and 1:160) and in 2 captive animals: a mature ewe (1:80) and a hybrid ram (1:160). None of the 5 animals carrying significant titers were showing signs of pneumonia at the time the blood samples were drawn. No neutralizing antibodies against IBR virus were demonstrated. All tissue culture results were negative for CPE or hemadsorption.

Bacteria resembling *Pasteurella* sp. brochemically and morphologically were isolated from the lungs of the 3 captive animals which had pneumonia. All bacterial cultures from nasal swabs were negative for similar bacteria.

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DISCUSSION

It is of interest that the hybrid ram carrying the 1:160 titer against SF-4 virus had been placed in a pasture with 16 bighorn ewes and lambs 2 weeks prior to the occurrence of pneumonia in the ewes. This resulted in the eventual loss of the entire group of bighorns. The bighorns had been trapped from the wild and released into the pasture 8 months prior to the outbreak.

Domestic livestock inhabiting the summer and winter ranges of the wild sheep during the summer could provide a source of infection of SF-4 virus or other pathogenic organisms. Pathogens may have been introduced into the captive herd either by contact with infected live-trapped bighorns, humans who were intermediate carriers, or domestic calves and sheep inhabiting nearby corrals and pastures.

Mule deer in Colorado had antibodies against IBR virus and were found to be susceptible to experimental infection (Chow and Davis, 1964). Wild deer have been reported to have HI antibodies against both the bovine and human strains of myxovirus parainfluenza-3 (Shah, *et al.*, 1965; Woods and Marquis, unpublished), and against bovine virus diarrhea (Kahrs *et al*, 1964). Wild reindeer in Sweden and a captive herd in the United States showed no antibodies against the virus (Bakos and Dinter, 1960; Bolton

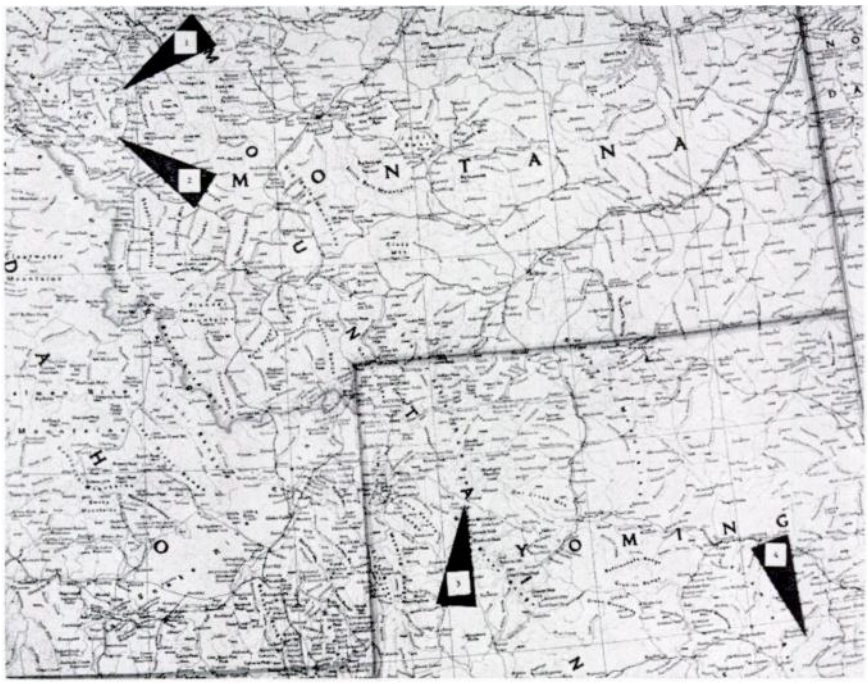

Figure 1. *Locations of bighorn sheep that were examined for evidence of respiratory virus infections* (1) *Wildhorse Island,* (2) *National Bison Range,* (3) *Wind River Mountain trap site,* (4) *Sybille Experiment Unit.*

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and Murray, 1964). A California research worker was not able to demonstrate viral agents from specimens of lungs from bighorn sheep killed by hunters or from adult specimens the lungworm of *P. rushi* (Forrester, 1965).

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