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Response of Vaccinated and Unvaccinated Bighorn Sheep (*Ovis canadensis canadensis*) to Experimental Respiratory Syncytial Virus Challenge

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ABSTRACT: Five Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*), approximately 5 mo old and without detectable antibody titers to respiratory syncytial virus (RSV), were assigned to two groups to study the effects of RSV challenge inoculation in vaccinated (n = 3) and unvaccinated (n = 2) bighorns. The three lambs vaccinated with a modified live bovine RSV vaccine developed a detectable antibody response to the vaccine. Vaccinated and unvaccinated lambs challenged with an ovine isolate of RSV developed increased levels of neutralizing antibody, but clinical signs of disease were not observed. Neutralizing antibody titers to RSV remained higher (2–4-fold) in vaccinated lambs over time when compared to unvaccinated lambs.

Key words: Bighorn sheep, *Ovis canadensis canadensis*, vaccine, respiratory syncytial virus, serum antibody titer, experimental study.

Pneumonia is the major cause of mortality in free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) in North America (Buechner, 1960). Lungworms (Buechner, 1960; Clark et al., 1985), bacteria (Post, 1962; Woolf et al., 1970), and viruses (Parks et al., 1972; Parks and England, 1974) have been isolated from bighorn sheep in fatal epizootics, and thus are implicated as the major etiologic agents in the pneumonia complex.

Respiratory syncytial virus (RSV) has been determined to cause acute pneumonia in domestic cattle (Rosenquist, 1974; Bryson et al., 1983), and has been implicated also as a predisposing agent to secondary bacterial pneumonia in domestic cattle (Thomas et al., 1980), and more recently in sheep (Trigo et al., 1984; Evermann et al., 1985). A RSV isolate has been reported from a bighorn sheep lamb (Spraker and Collins, 1986), and recently from three of four 6-mo-old Rocky Mountain bighorn sheep that died in an epizootic of pneumonia involving all ages of hosts in Oregon (Washington Disease Diagnostic Laboratory, Pullman, Washington 99164, USA; case number 86-10819). However, the role of RSV in the respiratory disease complex of free-ranging bighorn sheep is unknown. The purpose of this research was to evaluate the serologic response of bighorn sheep to a modified live virus vaccine for bovine RSV before and after challenge with an ovine isolate of RSV, and to evaluate the effects of a challenge inoculation of ovine origin RSV in vaccinated and unvaccinated bighorn sheep lambs.

Five 5-mo-old Rocky Mountain bighorn sheep which were born in a captive herd located at Washington State University (Pullman, Washington 99164, USA), were removed from their dams and acclimated to indoor-outdoor housing for 1 wk prior to beginning the experiment. The pens consisted of an indoor room (3.5 × 3.5 m) with pine shavings for bedding, and an outdoor run facility (3.5 × 3.5 m) with concrete floors. Pens were separated by concrete dividers. Each lamb was housed individually for the first 124 days of the 295 day experiment. The lambs had access to free-choice alfalfa pellets, alfalfa hay, water, and mineralized salt. They were observed at least twice daily. Lambs were divided into treatment groups of three vaccinated and two unvaccinated controls. Lambs were vaccinated intramuscularly with 2 ml modified live bovine RSV (BRSV)

vaccine (Bovine Respiratory Syncytial Virus Vaccine, Norden Laboratories, Inc., Lincoln, Nebraska 68501, USA) on days 0, 16, and 90 of the experiment. Blood was collected from all five lambs on each vaccination date for evaluation of RSV serum virus neutralizing antibody titers. On day 97 of the experiment, all five lambs were challenged with 20 ml of 1×10^5 tissue culture infective doses (TCID) 50/ml ovine isolate of RSV (ORSV, WSU 83-1578; Washington State University, Pullman, Washington 99164, USA). The virus suspension was administered orally (10 ml), intranasally (8 ml), and topically to the conjunctival sac (2 ml) to each lamb.

The ovine RSV was initially isolated from a domestic sheep with rhinitis (Evermann et al., 1985), and amplified in cell culture using ovine fetal tracheal cells. Cells were grown in Eagle's Minimal Essential Medium (Gibco Laboratories, Grand Island, New York 14072, USA), with Eagle's Salt Base supplemented with 10% heat-inactivated (56 C for 1 hr) fetal bovine serum (FBS) free of bovine viral diarrhea (BVD) virus and BVD viral antibodies, and gentamycin was added to the growth media at a concentration of 50 μg/ml. Cells were incubated at 37 C and the virus harvested when 3+ cytopathic effect (CPE) was achieved in cell culture.

Blood was collected from all lambs at the time of virus challenge (day 97), and also on day 124 (27 days after challenge) for evaluation of RSV serum virus neutralizing antibody titers before and after virus challenge. On day 124, all lambs were released from confinement into a 2-ha fenced pasture. Blood was collected on day 295 to evaluate RSV antibody titers over time.

A microtiter virus neutralization test was used for serological analysis (LeaMaster et al., 1984). A bovine strain of RSV (Mohanty A51908; originally isolated by S. Mohanty, Virginia Polytechnic Institute, Blacksburg, Virginia 24061, USA) was used as challenge virus in vitro. Initially, each serum sample was diluted 1:5 in Eagle's Minimum Essential Medium (MEM) without FBS, and heat-inactivated at 56 C for 30 min. Serial 2-fold dilutions were performed using semi-automated diluters (Titertek, Flow Laboratories, Inc., McLean, Virginia 22102, USA). The serologic tests were conducted in bovine turbinate cells obtained from the National Veterinary Services Laboratory (Ames, Iowa 50010, USA) which were grown in MEM with Earle's Salt Base supplemented with 10% heat-inactivated FBS. Gentamycin (Gentamycin reagent solution, Flow Laboratories, Inc., McLean, Virginia 22102, USA) was added to the medium at a final concentration of 50 μg/ml. The microtiter plates were incubated at 37 C in 5% CO_2 for 5 days. The virus neutralizing antibody titers were expressed as the highest dilution of serum that prevented 50% RSV cytopathogenic effect.

Lambs vaccinated with BRSV vaccine developed serum antibody titers of 1:80 to 1:160 (Table 1). Antibody titers increased after challenge with ORSV but clinical signs to the virus challenge were not observed in either vaccinated or unvaccinated animals. Antibody titers to RSV of 1:80 were detected in unvaccinated lambs 27 days postchallenge (experimental day 124) with ORSV. Although the level of antibody was less than that of vaccinated lambs, there was a notable response to the virus (Table 1). Antibody titers to RSV were markedly lower 198 days postchallenge, especially in the unvaccinated lambs (Table 1). At the termination of the experiment, antibody titers to RSV were 2–4-fold greater in vaccinated lambs (1:20 to 1:40) than in unvaccinated lambs (1:10).

Respiratory syncytial virus has been identified as an important respiratory pathogen in humans (Chanock and Parrot, 1986), cattle (Rosenquist, 1974; Bryson et al., 1983), and possibly sheep (Trigo et al., 1984; Evermann et al., 1985) throughout the world. It is theorized that the pathogenic mechanism utilized by RSV is destruction of the ciliated epithelium of the bronchus and bronchioles within the lung;

TABLE 1. Serum neutralizing antibody titers against respiratory syncytial virus (RSV) in vaccinated and unvaccinated bighorn sheep lambs (*Ovis canadensis canadensis*).

	Vaccination 1 Day 0	Vaccination 2 Day 16	Vaccination 3 Day 90	Virus challenge Day 97	Day 124	Day 294
Vaccinates						
Lamb 1	<5[a]	40	5	160	160	20
Lamb 2	<5	40	10	80	80	40
Lamb 3	<5	40	5	80	320	40
Unvaccinated controls						
Lamb 4	<5	<5	<5	<5	80	10
Lamb 5	<5	<5	<5	<5	80	10

[a] Titers expressed as the reciprocal of the highest dilution of serum causing 50% reduction in RSV cytopathogenic effect.

thus, mucocilliary clearance of the lung is impaired, and infection by opportunist secondary bacteria such as *Pasteurella* spp. is enhanced (Eis, 1979). Respiratory syncytial virus has been demonstrated as the sole cause of acute fatal pneumonia (Thomas et al., 1980), and in conjunction with *Pasteurella* spp. infection in cattle (Rosenquist, 1974). Since RSV isolates from different species are antigenically similar, the potential for interspecies transmission has been hypothesized (Thomas et al., 1984; Evermann et al., 1985). Recent studies have shown that interspecies transmission can occur under experimental conditions (W. J. Foreyt, pers. obs.). Based on these studies, it was theorized that RSV may be involved in acute fatal pneumonia outbreaks in wild bighorn sheep. Sera collected from bighorn sheep in nine western states (USA) revealed that 42% had detectable neutralizing antibody titers to RSV (Dunbar et al., 1985), indicating that the virus was circulating in free-ranging sheep. However, the importance of the virus as a primary pathogen or as a predisposing agent to respiratory disease remains unknown.

In this experiment, three bighorn sheep lambs were vaccinated with a commercially available bovine origin RSV vaccine and all developed high titers of neutralizing antibody to RSV antigen, indicating that the vaccine elicited a response that may be adequate to prevent subsequent infection with RSV. When these lambs were challenged with a sheep isolate of RSV, their antibody titers increased 2–4-fold.

When all five bighorn lambs were challenged with the domestic sheep isolate of ORSV, clinical signs of disease were not noted in either vaccinated or unvaccinated individuals. These preliminary data may indicate that the domestic sheep isolate of ORSV, at the levels used, was not pathogenic under our defined conditions (Adair and McFerran, 1987). Further studies on the virulence of bighorn sheep RSV isolate (WSU 86-10819) are needed to resolve the question of strain variation in the disease process. All five lambs demonstrated an antibody response. Antibodies in all five lambs persisted 197 days after challenge with the virus, but at low levels. Antibody titers in vaccinated sheep remained 2–4-fold higher over time when compared to unvaccinated sheep, indicating that the modified live vaccine may have potential value in preventing recurrent infections in bighorn sheep. Serological evidence indicates that RSV may be widespread in bighorn populations (Dunbar et al., 1985); thus, the possibility of RSV being important in the bighorn sheep pneumonia complex exists, especially when conditions where physical and etiological agent stressors influence the health of bighorns. Further studies with ORSV alone and in conjunction with other agents associated with respiratory disease are warranted.

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