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## VACCINATION TRIALS IN DESERT BIGHORN SHEEP AGAINST BLUETONGUE VIRUS\*

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**Abstract:** Immunization of desert bighorn sheep (*Ovis canadensis*) against bluetongue virus was attempted by inoculation of live virus vaccine with a syringe and needle, by experimentally contaminated *Culicoides* gnats, the natural biological vectors, by bites of exposed *Stomoxys calcitrans*, which were considered to be natural mechanical vectors, and by incorporation of lyophilized vaccine in ground dry feed. Only the methods using the syringe and needle and the natural biological vector were effective.

### INTRODUCTION

Bluetongue virus has been isolated from Texas desert bighorn sheep in connection with losses in a semi-captive herd in Brewster County.<sup>4</sup> Bluetongue was thought to be a potential limiting factor in the re-introduction of this game species into Texas Trans-Pecos rangelands. Clinical cases of bluetongue also have been found to be widespread in Texas white-tailed deer,<sup>5,6</sup> thus indicating the chance for transmission of this disease from deer to bighorn sheep.

The fact that vaccine bluetongue virus can be transmitted from ill to normal sheep by the bite of contaminated laboratory-reared *Culicoides*,<sup>1</sup> suggested the possibility of using this gnat to transmit vaccine virus to free-living wild animals. Since the disease has been widespread in domestic sheep for some time,<sup>2,3</sup> vaccination appears to be the most likely means of control of this disease in bighorn sheep.

### METHODS

Desert bighorn lambs were captured and transported to the Wildlife Disease Laboratory, Texas A&M University, where they were reared to the age of 6-18 months. The difficulty of obtaining this rare species limited the size and number of experiments in that only one or two animals were available each year.

#### Trial I:

The first trial consisted of using a syringe and needle to inoculate a bighorn sheep with 2 ml of commercially available bluetongue vaccine<sup>†</sup> to evaluate the effect of this agent on bighorn sheep. Body temperatures were recorded daily, and standard blood values (packed cell volume, total leukocyte counts, differential leukocyte counts, thrombocyte counts) were determined. Inoculation with 10 ml field bluetongue virus in

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† Blucine: Cutter Laboratories, Berkeley, California.

whole blood was performed to determine if the bighorn sheep was actually resistant to challenge with field virus.

#### Trial II:

The second bighorn sheep was inoculated against bluetongue by the following method: a serologically negative bluetongue-free domestic sheep was inoculated with bluetongue vaccine. Four days following vaccination, a laboratory culture of *Culicoides varipennis* was allowed to take a blood meal from this domestic sheep.

The gnats were then rested and allowed to oviposit, when they were again allowed to feed, this time on the experimental desert bighorn sheep. Following the gnat feeding, standard blood determinations were made on the desert bighorn. Two weeks following inoculation, the bighorn sheep was challenged with field virus in a similar manner to the first trial using the same inoculum to determine if immunity had been conferred.

#### Trial III:

Two bighorn sheep were utilized. An attempt was made to confer immunity against bluetongue by incorporating 0.128 g lyophilized bluetongue vaccine in 180 g dry feed. The bighorns were hand-fed immediately following mixing and standard blood values were followed through a 2-week period. Serological studies utilizing the agar gel diffusion technique were then done to determine if any immunity had been conferred.

#### Trial IV:

A serologically negative bluetongue-susceptible domestic sheep was inoculated with bluetongue vaccine. A laboratory culture of *Stomoxys calcitrans* was allowed to feed on this animal on the 4th and 5th days post-inoculation. The following day the flies were allowed to feed on two desert bighorn sheep. The sheep were bled daily and standard blood determinations were performed to find suggestive evidence of a vaccination "take". Two weeks following exposure to the flies, a blood sample from each ex-

perimental bighorn was analyzed for presence of antibody against bluetongue virus, using the agar gel diffusion technique.

### RESULTS

#### Trial I:

Inoculation of a desert bighorn sheep against bluetongue using a commercially available vaccine produced immunity and did not result in clinical illness. The most significant blood value determined was the thrombocyte count. The thrombocyte counts dropped precipitously 7 days following inoculation of bluetongue vaccine from a base value of 834,000/mm<sup>3</sup> to 366,000/mm<sup>3</sup> and did not regain pre-inoculation values for 29 days. When this animal was challenged with field virus, no clinical illness resulted.

#### Trial II:

Inoculation of a desert bighorn sheep against bluetongue using the natural vector, *Culicoides varipennis*, was effective. Thrombocyte counts dropped 5 days following gnat inoculation, from a base value of 578,000/mm<sup>3</sup> to 246,000/mm<sup>3</sup> and the animal had no clinical illness following challenge with field virus. Vaccination was accomplished by the feeding of gnats on the experimental host.

Cultures of *Culicoides* were difficult to manage due to their small size and the fact that the females would not repeat a blood meal until oviposition took place. Mortality in the *Culicoides* culture was high.

#### Trial III:

There was no serological evidence of immunity following the feeding of lyophilized vaccine in dry feed. Blood values in the test animals did not vary significantly from pre-trial values.

#### Trial IV:

There was no serological evidence that immunity was conferred by the bites of *Stomoxys calcitrans*. Blood values did not

vary significantly from pre-inoculation samples. *S. calcitrans* was an extremely hardy vector and fed avidly on any host proffered. Mortality in the vector culture was low and the flies were capable of living for considerable periods without a blood meal—a factor that makes them attractive as an experimental vector.

#### DISCUSSION

Immunization of desert bighorn sheep against bluetongue appears possible in two ways: direct inoculation and the use of the natural biological vector. The use of direct inoculation is the most reliable means of producing immunity, but its use is limited in free ranging populations due to difficulty in approaching the animals, and the limited range of projectile syringe guns presently available. The use of this method of immunization is restricted to animals that are readily approached or individuals that are captured and actually handled.

Free ranging populations could conceivably be vaccinated using the natural vector; however, several disadvantages should be considered before such means are employed. First, inoculation of all members of a population would be unlikely; as in most sheep populations, some animals are not present in groups at all times; thus the aim would be to vaccinate the majority of the population rather than all members. Second, the possibility that vector passage of a vaccine virus may increase the virulence of that virus must be considered. This would dictate that vaccine strains of virus should be avirulent field isolates rather than laboratory attenuated viral strains which may be more likely to increase in virulence. Third, the release of vaccine laden vectors should be made at a time when repeated passage of the vaccine virus is unlikely; i.e., the vectors are released at a time of year or in an unfavorable environment in which vector reproduction is unlikely or at a minimum. Fourth, artificial propagation of *Culicoides* was found to require considerable attention. Once the female gnats had fed on the vaccinated domestic sheep, they would

not feed again until oviposition had taken place. This required constant surveillance of the gnat cultures, because the gnats died soon after oviposition if not given an opportunity to feed. Considerable mortality appears to be the rule in utilizing this species for vaccination purposes.

Trials using a passive vaccination (in dry feeds) or using the much more robust stable fly, *S. calcitrans* (a mechanical vector) failed to stimulate immunity to the bighorn sheep in this pilot study, thus suggesting that the biological vector is required in the transfer of bluetongue virus in bighorn sheep.

The use of natural vectors in the management of disease in wild populations warrants consideration. From these limited trials, the advantages of using *Culicoides* as "mobile syringes" to seek out and vaccinate animals that cannot be approached by humans are not difficult to envision; and the potential of using similar systems in other game species is great. Incorporation of vaccines in feed or the use of more easily handled vectors also bears investigation.

In this pilot study, oral vaccination in feed was explored due to its ease of application in the field. The mechanical vector, *S. calcitrans*, was also investigated because of its hardiness and ease of handling in the field. Unfortunately, neither of these methods were effective.

Since the initiation of these pilot studies, inoculation of desert bighorn sheep with vaccine has been done by hand or with projectile syringes whenever opportunity presented. Vaccination by projectile-syringe was performed during attempts to control a die-off on the Black Gap Wildlife Management Area<sup>2</sup> and mortalities attributable to this procedure did not occur. All bighorn sheep moved to the Sierra Diablo Wildlife Management Area have been vaccinated manually by syringe and this procedure also produced no mortality or illness in the animals.

Therefore, it is the opinion of the authors that in areas where bluetongue is a proven problem in bighorn sheep, vaccination is indeed possible, even mandatory in the management of this species.

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