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Bluetongue Virus in North American Elk*

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Abstract

North American elk *(Cervus canadensis)* were susceptible to experimental bluetongue virus (BTV) infection although clinical signs were mild or inapparent. A viremia of significant magnitude and duration occured in all five experimental elk following subcutaneous inoculation. Elk developed BTV antibody by the second or third week after exposure and antibody was still present in the sera of all animals at the termination of the experiment at 6 or 7 months. The possible role of elk in the epizootiology of bluetongue was discussed.

Knowledge of bluetongue in wild ruminants of North America is limited and until recently there was little evidence of their involvement in bluetongue epizootiology. The white-tailed deer *(Odocoileus virginanus)* has been shown to be susceptible to BTV under experimental and captive condition.[4,5,7] In addition, Robinson *et al*[2] reported a natural case of bluetongue in a bighorn sheep *(Ovis canadensis)* in Texas.

A recent serologic study of wild ruminants in North America reported the detection of BTV antibody in a variety of wild ruminants.[6] Among the species listed as serologic reactors were North American elk. Because of the overlapping geographic distribution of bluetongue and elk, the common use of range by sheep and elk, and the detection of BTV serologic reactors in elk, this study was undertaken to determine the susceptibility and response of elk to BTV.

Materials and Methods

Virus: The virus used as inoculum in these studies was BT8, the "standard" North American strain of BTV. It was originally obtained in sheep blood from Dr. J. G. Bowne, U.S.D.A. - A.R.S., Bluetongue Research Laboratory, Denver, Colorado and was subsequently passed in several white-tailed deer.[7] Blood from one of these deer (D 6) containing approximately 10^4 tissue culture infective $doses_{50}$ ($TCID_{50}$) as determined by titration in L cells was used as inoculum.

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Experimental Animals: The experimental animals were North American elk obtained either from a commercial "game farm" located at Beaver Dam, Wisconsin or as offspring born to these animals at the University of Wisconsin Charmany Research Center. The experiment involved five animals: E-1, a yearling female; E-2, a six month old female; E-3, a mature female; and two four-month old males, E-60 and E-102, which were born and reared at the Charmany Center.

Experimental Procedure: All animal experiments were done in Rockefeller type isolation buildings. Individual elk were placed in isolation units approximately one week prior to inoculation, during which time reference temperatures, serologic data, and hematologic values were obtained. For a two week period following subcutaneous inoculation with 10^4 $TCID_{50}$ of BTV, animals were observed twice daily to detect clinical evidence of disease, temperatures were recorded, and daily blood sampls were collected for routine hematology and virus isolation. Serum was collected from each animal at weekly intervals for 6 months.

On day post inoculation (DPI) 105, animals E-60 and E-102 each received a 5 mg. intramuscular injection of cortisone (Flumethasone, Syntex Laboratories, Palo Alto, California). Following this injection, daily observations were made to detect clinical evidence of disease and blood samples for virus isolation were collected for 5 days.

On DPI 131, animals E-1 and E-2 were inoculated subcutaneously with 5 ml of blood from a deer which had died from Epizootic Hemorrhagic Disease (EHD). For one week, daily observations were made to detect clinical evidence of disease and blood specimens were collected for virus isolation.

Blood for virus isolation attempts was routinely collected by jugular puncture, mixed 50:50 with oxalate-phenol-glycerin (OPG), and held at 4°C until tested. Virus isolation attempts were made in monolayer cell cultures of mouse L cells (L-929 Grand Island Biological Company, Grand Island, New York) utilizing the methodology of Thomas and Trainer.[5] Individual 0.1 ml samples of the 1:10 dilution of blood in OPG were inoculated onto each of four drained monolayer cultures and observed for cytopathic effects (CPE). Isolated agents were considered BTV if they were specifically neutralized by reference BTV antiserum.

All serologic testing was conducted at the U.S.D.A. - A.R.S., Bluetongue Research Laboratory in Denver using the agar gel precipitin (AGP) technique described by Jochim and Chow.[1]

Hematologic values examined included total red blood cell counts, total white blood cell counts, differential white blood cell counts, hemoglobin and hematocrit.[3]

Results

Experimental exposure of elk to BTV resulted in a subclinical infection in animals E-1, E-2, and E-3. The two 4-month old elk calves, E-60 and E-102, had mild conjunctivitis and diarrhea containing small amounts of blood and mucous on DPI 9 and 10. A transient rise in temperature was observed DPI 5 to 9 in all animals except adult cow, E-3. The only hematologic change detected was a leukopenia which each animal experienced from DPI 2 to 10. A "typical" temperature and leukocyte response is illustrated in Figure 1.

BTV was isolated from the blood of all elk following experimental exposure (Table 1). A viremia occurred on DPI 2 in elk E-1 and E-2, and persisted through DPI 10 in elk E-2. BTV was isolated from all experimental animals on DPI 7 and 8. The viremia titer ranged from 3.4 to 4.3 $TCID_{50}$/ml of blood and appeared to peak on DPI 7.

BTV was isolated from the blood of elk E-60 and E-102 collected on day 2 and 3 following the Flumethasone injection.

FIGURE 1

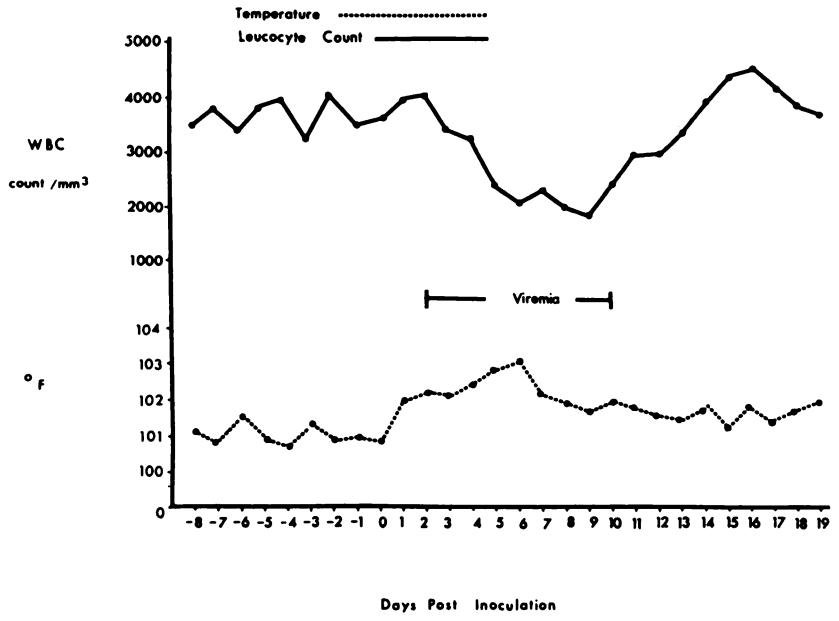

FIGURE 1. *A typical leukocyte, viremia and temperature response of elk to experimental bluetongue virus infection (elk E-2).*

A serologic response as measured by the AGP test occurred in all elk sera following inoculation with BTV. The first evidence of BTV antibody occurred at 2 weeks post inoculation in animals E-3 and E-102. Elk E-1 and E-60 converted serologically at 3 weeks and elk E-2 at 5 weeks. Elk E-1 and E-2 were still AGP positive when killed at 6 months post inoculation; all other elk were still reactors 7 months after infection (Table 2).

No clinical reaction was detected in elk E-60 or E-102 following inoculation with E.H.D. virus, but 2 to 6 weeks after E.H.D. challenge a "very strong AGP test reaction" for BTV antibody was reported by Dr. M. M. Jochim, U.S.D.A.-A.R.S., Bluetongue Research Laboratory, Denver.

TABLE 1. *Summary of viremia studies of elk following experimental bluetongue virus infection.*

Elk No.	Days Post Inoculation*																	
	0	1	2	3	4	5	6	7	8	9	10	12	14**	105**	106	107	108	109
E-1	—	—	+	—	+	+	+	+	+	+	—	—	—	•	•	•	•	•
E-2	—	—	+	—	—	+	+	+	+	—	+	—	—	•	•	•	•	•
E-3	—	—	—	—	—	—	—	+	+	+	—	—	—	•	•	•	•	•
E-60	—	—	—	+	+	•	+	+	+	+	—	—	—	—	+	+	—	—
E-102	—	—	—	+	+	•	+	+	+	—	—	—	—	—	+	+	—	—

* a + indicates BTV was isolated; a — indicates no BTV was isolated; a • indicates not tested.
* All virus isolation attempts were negative between DPI 14 and 105; On DPI 105 elk E-60 and E-102 were injected with 5.0 mg. Flumethasone.

TABLE 2. *Summary of agar gel precipitin test results of elk following experimental bluetongue virus infection.*

Elk No.	Weeks post inoculation*										Months post inoculation*				
	—1	0	1	2	3	4	5	6	7	8	3	4**	5	6	7
E-1	—	—	—	—	+	+	+	+	+	+	•	+	X	X	•
E-2	—	—	—	—	—	—	+	•	•	+	•	—	X	X	•
E-3	—	—	—	+	—	+	+	•	•	+	+	+	—	+	+
E-60	—	—	—	—	+	+	+	•	•	+	+	+	+	+	+
E-102	—	—	—	+	+	+	+	•	•	+	+	+	+	+	+

* a + indicates a AGP reactor; a X indicates a strong reactor; a — indicates a non-reactor; a • indicates not tested.
** EHD virus was inoculated in elk E-1 and E-2 on day 131.

Discussion

Results of the experimental exposure studies indicated that elk of both sexes and different ages were susceptible to BTV infection. Clinical response was mild in young animals and inapparent in adult elk. A viremia occurred in elk 2 to 10 days following exposure. BTV antibody was detected in all elk following exposure and it persisted until the end of the experiment, 6-7 months post inoculation.

Because of the similarities of EHD and bluetongue in white-tailed deer[6,7] two elk which had previously been exposed to BTV were challenged with EHD virus. Although there was no clinical response to the EHD virus challenge, a strong AGP test reaction resulted. Although the AGP test is not quantitative, the sera collected 2-6 weeks after EHD virus exposure produced the strongest reaction of any elk sera examined by Dr. M. M. Jochim (personal communications). An explanation of this result is not available; although, the possibility of an anamnestic response is interesting. The relationship of EHD to BTV remains unkown, and these findings further illustrate the importance of clarifying this subject.

The isolatino of BTV from two elk immediately following an injection of Flumethasone could be of significance. The restimulation of a viremia with cortisone could theoretically provide a mechanism which might perpetuate arthropod transmission. The potential of elk as a bluetongue reservoir merits further investigation.

The overlapping geographic distribution of BTV and elk, the similar grazing ranges of livestock and elk, the serologic evidence of BTV in elk, and the susceptibility of elk to BTV, all are suggestive that BTV exists naturally in elk. Additional research with this wild ruminant species is important to further clarify its role in bluetongue epizootiology.

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