Bluetongue Virus: (1) In Pregnant White-tailed Deer (2) A Plaque Reduction Neutralization Test*

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Bluetongue Virus: (1) In Pregnant White-tailed Deer (2) A Plaque Reduction Neutralization Test*

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Abstract

Six white-tailed deer *(Odocoileus virginianus)* were infected with bluetongue virus (BT_8, vaccinal strain) approximately one-third of the way through their gestation period. One deer died of bluetongue 21 days after inoculation. Of the five surviving the infection, one had two mummified fetuses, and the others no fetuses upon euthanasia two weeks after term. Fetuses were present in two control deer and in the one which died of bluetongue. A plaque reduction neutralization test for bluetongue virus was developed and described for the first time and its sensitivity illustrated by high post inoculation titers which ranged from 1:3200 to greater than 1:16000.

Introduction

Bluetongue virus (BTV) infection of deer under both natural[10,12] and experimental conditions[11] has been described. In addition, BTV has been shown to cause congenital infection of sheep and fetal abnormalities.[2,4,9,12] The following experiment was done to determine if a similar effect occurs in white-tailed deer infected during gestation with BTV.

A sensitive quantitative method for detecting BTV antibodies has not previously been available, with the possible exception of the complement fixation test.[1] A plaque reduction test was developed in the present investigation to demonstrate the magnitude of antibody response and to provide a more sensitive technique for future application.

Materials and Methods

Experimental Animals:

Eight adult female white-tailed deer were used in the experiment. These were part of a captive deer herd which has been maintained at the Charmany Research Center, University of Wisconsin, for several years and whose reproductive history has been consistently good (i.e. averaging nearly 2 live fawns per bred doe annually). All had been allowed to breed during the previous rut and appeared normal at the time of inoculation, approximately one-third of the way through their gestation period. Deer which died during the experiment were necropsied, tissues being taken for virus isolation

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TABLE 1. *Experimental protocol and results of inoculation of pregnant white-tailed deer with bluetongue virus.*

	Deer No.	Inoculum*	Results	Neutralizing Antibody Titer** Preinoculation	At Term	Additional Findings
Group I	92	10^5 $TCID_{50}$	Barren at term	1/16	1/16,000	No gross abnormalities
	95	10^5 $TCID_{50}$	Barren at term	1/7	1/10,000	No gross abnormalities
	38	10^5 $TCID_{50}$	Barren at term	1/16	1/4,400	No gross abnormalities
Group II	45	10^5 $TCID_{50}$	Barren at term	1/5	1/6,900	No gross abnormalities
	49	10^5 $TCID_{50}$	2 Mummified fetuses	1/5	1/3,200	Mummified fetuses sterile
	47	10^5 $TCID_{50}$	Died PID 21, bluetongue	1/5	NT***	2 fetuses — virus isolation from placenta and lung only —(gross lesions characteristic of bluetongue).
Group III	77	Diluent	2 normal fawns at term	1/16	1/22	No observed abnormalities
	44	Diluent	Died PID 28, undetermined cause	1/17	NT	2 fetuses — no virus isolation and no lesions characteristic of bluetongue

* Vaccinal strain BT_3 titrated in L-929 cells.

** Plaque reduction titers.

*** Not tested.

TABLE 2. *Bluetongue isolation attempts* from inoculated deer.*

Deer Number	History	Tissue	Results
47	10^3 $TCID_{50}$, died PID 21, gross lesions bluetongue.	Spleen	Neg.
		Blood	Neg.
		Lung	Pos.
		Uterus	Neg.
		Placenta	Pos.
		Fetuses	Neg.
44	Contact control, died PID 28, undetermined cause.	Spleen	Neg.
		Blood	Neg.
		Lung	Neg.
		Placenta	Neg.
		Fetuses	Neg.
49	10^3 $TCID_{50}$, euthanatized 2 weeks after term. 2 mummified fetuses.	Placenta	Neg.
		Fetuses	Neg.
92, 95, 38	10^5 $TCID_{50}$, euthanatized 2 weeks after term.	Uterus	Neg.
		Ovary	Neg.
45	10^3 $TCID_{50}$, euthanatized 2 weeks after term.	Uterus	Neg.
		Ovary	Neg.

* Isolation attempts in L-929 cells.

attempts and serum for antibody titer. All deer surviving the experiment were killed and necropsied approximately two weeks after term.

Virus:

Two dilutions of BT_8 vaccinal[1] strain, were prepared in diluent (Hanks Balanced Salt Solution plus 10% fetal bovine serum) containing approximately 10^5 and 10^3 $TCID_{50}$ per ml. The previously described[11] L cell system was used to titrate the inocula and to attempt BTV recovery from tissues (Tables 1 and 2). Virus recovery from animals which died during the experiment was attempted from the spleen, lung, blood, placenta, and fetuses. The ovaries, uteruses and contents (when present) from deer euthanatized at term were similarly processed (Table 2).

Inoculation Procedure:

The deer were paralyzed with succinylcholine[2] using a CO_2 "Chap-chur" gun and inoculated subcutaneously. The deer in group I, no.'s 92, 95, & 38, were given 10^5 $TCID_{50}$; the 3 deer in group II, no.'s 45, 49, & 47, were given 10^3 $TCID_{50}$; the 2 deer in group III, no.'s 77 and 44, were given diluent only.

Serology:

A previously undescribed plaque reduction neutralization test was used to measure antibodies against BTV. An isolate of BTV, Strain BT_8, was obtained from the U.S.D.A. bluetongue laboratory[3] in Denver, Colorado. It was subsequently cloned by three consecutive plaque passages using a modification of the plaquing system described by Howell,[5] and

[1] Blucine, Cutter Laboratories, Berkeley, California.
[2] Anectine, Burroughs Wellcome and Co., Inc., Tuckahoe, New York.
[3] United States Department of Agriculture, Animal Disease Research Laboratory, Building 45-B, Denver Federal Center, Denver, Colorado.

stocks prepared. Two fold dilutions of test sera were made and a suspension of virus containing approximately 60 plaque forming units (p.f.u.) per 0.1 ml was added to an equal volume of each serum dilution at 0°C. The mixtures were agitated, heated to 37°C in a water bath and held at this temperature for 1 hour. At the end of this incubation period, each serum virus mixture was inoculated in 0.2 ml volumes onto each of 3 washed monolayer cell cultures of L-929 cells in 60 x 15 mm plastic petri dishes.[4] These plates were held on incubator trays, each of which had 3 positive control plates. The latter had the serum dilution replaced by an equal volume of diluent. The trays were rocked at frequent intervals during the next hour to allow maximum absorption.

At the end of this incubation period, the plates were washed with diluent, drained, and overlaid with semi-solid medium. The latter consisted of 2X MEM and 1.6% agar[5] in a 1:1 ratio with 0.20 $\mu g''$/ml of diethylaminoethyl dextran[6] and 1% fetal bovine serum added. The overlay solidified in 5 to 10 minutes at room temperature. The plates were inverted and returned to the incubator.

On day 4 of their incubation the cells were stained by the addition of 1 ml of a 1:2000 dilution of neutral red[7] in growth medium. The plaques were counted 4 to 24 hours after staining. The number of plaques was averaged for each group of 3 plates and the percent reduction for each serum dilution was calculated by comparison with the control. The serum dilution causing the closest to 50% reduction and the one above and below were plotted on log probit paper as described by Russel *et al.*[8] Using these three points, the line of best fit was graphically estimated and the point of its intersection with the 50% vertical (5 probit) taken as the reduction titer (read on the ordinate).

Results

None of the deer inoculated with BTV produced fawns. The 3 deer receiving 100,000 $TCID_{50}$ remained apparently normal through term although none had any evidence of fetuses on necropsy. In the group of 3 receiving 1000 $TCID_{50}$, one deer, 47, died with typical lesions of bluetongue on post inoculation day (p.i.d.) 21. She had two grossly normal fetuses. BTV was isolated from the lung and placenta of the dam, but not from her blood or spleen nor from either of the two fetuses (Table 2). The other 2 deer in this group survived. Number 45 had no fetuses at necropsy while No. 49 had two mummified fetuses in the absence of other abnormal signs. Isolation attempts from the fetuses and placenta were negative (Table 2).

One of the two control deer, No. 77, had two normal fawns at term and had no rise in antibody titer. The other control, No. 44, died of unknown causes on PID 28. She had no gross evidence of BTV infection and attempts to isolate the virus from her blood, spleen, lung, placenta, and two fetuses were negative (Table 2).

The plaque reduction technique described, proved very sensitive as attested by the magnitude of the post infection titers which ranged from 1:3200 to greater than 1:16000 (Table 1). Preinoculation titers of 1:2 to 1:30 have been commonly observed in deer in our laboratory. It is assumed that this is a non-specific inhibition as described by Pini[7] for bovine sera. This assumption has not yet been tested.

Discussion

Unlike the observations in sheep, BTV in white-tailed deer appears to cause early absorption or uncomplicated abortion. The latter possibility is mentioned as the deer were kept in large outdoor pens and could conceivably have aborted without

[4] No. 3002 Tissue Culture Dish, Falcon Plastics, 5500 West 83rd Street, Los Angeles, California.
[5] Ionagar No. 2, Colab Laboratories Inc., Box 66, Chicago Heights, Illinois.
[6] DEAE-DEXTRAN, Pharmacia, Uppsala, Sweden.
[7] Grand Island Biological Co., 3175 Staley Road, Grand Island, New York.

being noticed. While it was not possible to determine if all animals were pregnant at the time of inoculation, it is nearly certain that all or most of them were, in light of previous fawning history of this herd, as well as the observed fetuses in the two deer that died, the surviving control and in a fourth doe in the herd which was killed for another reason. In summary, we were able to determine the pregnancy status in 4 of 9 does; each of these had 2 fetuses.

Because of the demonstrated natural occurrence of bluetongue in deer and their known susceptibility[10,11,12] it would be interesting to know what effect BTV has on the pregnant doe in nature. Results of this experiment suggest that it might easily go unnoticed inasmuch as live abnormal fawns seem not to be produced.

The plaque reduction neutralization test has long been recognized as one of the most sensitive methods of detecting antibodies.[8] The commonly used method of constant serum-varying virus combined with a quantal assay has been so severely criticized elsewhere as to make discussion of it unnecessary.[6,8] While the plaque reduction test is highly sensitive and quantitative, its limitations due to antigenic variation among isolates are as yet unknown. It appears to be a simple and very useful procedure for work with BTV.

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