Experimental Bluetongue Disease in White-Tailed Deer*

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ABSTRACT

Nine white-tailed deer and six sheep were experimentally exposed to the California BTV-8 strain of bluetongue virus. The infections were fatal for seven of the nine deer. An additional deer died from exposure to an isolate of bluetongue virus from bighorn sheep. Clinical signs and lesions of bluetongue in deer were described. The incubation period, signs and lesions of bluetongue and epizootic hemorrhagic disease of deer appear to be similar. Virus isolations were made from the blood and a variety of tissues of exposed deer and identified as bluetongue virus. Neutralizing antibodies were detected in all of the convalescent sera.

Introduction

Among domestic species, bluetongue is primarily a disease of sheep, although cattle and goats are susceptible (1).

Except for the blesbok (*Damaliscus albifrons*) and bighorn sheep (*Ovis canadensis*), the susceptibility and role of wild ruminants in this disease is unknown. Neitz (2) has shown that under experimental conditions the blesbok can develop an inapparent infection of bluetongue. A natural case of bluetongue in a bighorn ram in western Texas was documented by Robinson, *et al* (3). Bluetongue virus (BTV) has been detected in two species of wild rodents (*Rhabdomys pumila* and *Otomys irroratus*) (1). Suckling mice (4) and suckling hamsters (5) are the only laboratory animals susceptible to BTV. Information available on the host range of this virus in domestic and wild species appears to be limited to these reports.

The aim of this study was to determine the susceptibility of the white-tailed deer (Odocoileus virginianus) to BTV, and to study the experimental disease produced.

Materials and Methods

Virus. — The California BTV-8 strain of bluetongue virus, obtained as infective sheep blood from the Bluetongue Research Laboratory, USDA-ARS, Denver, Colorado was used in all but one of these experiments. The virus was preserved in oxalate-carbolic acid-glycerin solution (O. C. G.)[1] at 4°C. In this laboratory, the BTV-8 was passed serially in three sheep. Blood taken one day prior to the peak in temperature rise of either the second or third sheep passage was used as inoculum.

Bluetongue virus isolated from a bighorn sheep (3) and passaged two times in domestic sheep was obtained from R. M. Robinson, Texas A & M University[2]. This was used as the inoculum in one deer.

Experimental Animals. — White-tailed deer used in this study were obtained from the wild as fawns less than two weeks of age, were bottle fed and raised under penned conditions at the University of Wisconsin Charmany Research Center. Seven fawns, 7 to 8 months of age, (D35, D36, D2, D3, D4, D6 and D8) and three adults more than one year of age (D1, D5 and D7) were used in the experiments (Table I). All experimental studies were conducted during

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[1]O. C. G.: 500 ml. glycerin, 500 ml. distilled H_2O, 5 gm. potassium oxalate, 5 gm. phenol.

[2]The bluetongue viruses were obtained by permission of the ADE Division of the USDA under permit #174-T (Denver) and #2031 (Robinson).

the fall or winter months in a Rockefeller type isolation building (6). Deer were placed in isolation one week prior to exposure to obtain reference temperature and hematologic values.

The sheep (S695, S63, S1, S2 and S3) were mixed breeds approximately one year of age and obtained from central Wisconsin. Suckling mice, 2 to 3 days old, were obtained from the mouse colony maintained by the Department of Veterinary Science, University of Wisconsin. One day old embryonated chicken eggs were purchased from local hatcheries and incubated to the desired age for inoculation.

Experimental Design. — The protocol of the experimental inoculations is summarized in Figure I. For all inoculations, 2.5 ml. of the inoculum was injected intravenously and 2.5 ml. intramuscularly (Table I). Three deer (D36, D1 and D2) received blood from sheep (S63), the third passage of BTV-8 in domestic sheep in our laboratory. D35 was not inoculated, but shared an isolation room with D36 and died eight days after the death of D36. From D35 a 10% suspension of spleen in phosphate-buffered-saline (PBS) was prepared and filtered through a 0.2 u membrane filter. D3 and S1 received this filtered spleen suspension. D4 and S2 were inoculated with a similar 10% suspension of spleen from D35 that had not been filtered.

One deer (D5) was inoculated with blood from sheep (S695), the second passage of BTV-8 in domestic sheep in our laboratory. Two deer (D6 and D7) were inoculated with a 10% suspension of spleen from D2 which had succumbed to previous experimental exposure. The suspension of spleen contained 1,000 units of penicillin and 1 mg. of streptomycin per ml. of diluent. S3 was inoculated with blood taken from D2 one day prior to the peak of the temperature rise. D8 was inoculated with blood from the second sheep passage of the BTV isolated from the bighorn sheep.

All animals were examined for signs of disease and rectal temperatures were recorded daily. Five ml. of blood were taken either daily or every other day, preserved in an equal volume of O. C. G. and stored at 4°C until tested for the presence of BTV. Additional blood was collected for hematologic and serologic studies.

The extent and nature of gross lesions were recorded at necropsy and representative tissues were collected, fixed in formalin, and stained with hematoxylin and eosin. Duplicated tissue sections were frozen at −60°C until they could be tested for the presence of virus.

Virus Isolation Studies. — Deer and sheep blood collected during the study was diluted 10^{-1} and 10^{-2} in PBS, containing 0.15% lipase (7). Tissue specimens were ground in Ten Broeck tissue homegenizers to a 10% suspension in PBS containing 1,000 units of penicillin and 1 mg. of streptomycin per ml. The suspension was clari-

TABLE I. — Summary of Experimental Exposures of Deer and Sheep to Bluetongue (BTV-8)

EXPERIMENTAL ANIMALS*				EXPERIMENTAL RESULTS** (days post inoculation)		
Number	Age	Sex	Inoculum	Onset signs	Death	Temperature peak
S63	A	M	S695 Blood	0	.	
S1	A	M	D35 Spleen (filtered)	7	.	7 (106.8)
S2	A	M	D35 Spleen (unfiltered)	6	.	6 (106.0)
S3	A	M	D2 Blood	6	.	6 (106.8)
D36	F	M	S63 Blood	4	5	4 (104.7)
D35	F	M	Contact	5	7	U (103.6)
D2	F	M	S63 Blood	7	8	5 (106.0)
D3	F	M	D35 Spleen (filtered)	6	8	6 (104.8)
D4	F	M	D35 Spleen (unfiltered)	4	5	2 (104.8)
D6	F	M	D2 Spleen	7	8	5 (103.6)
D8	F	M	BS	8	8	7 (103.5)
D1	A	M	S63 Blood	0	.	5 (105.2)
D5	A	M	S695 Blood	0	.	6 (103.6)
D7	A	Fe	D2 Spleen	8	8	U

*S = Sheep, D = Deer, A = Adult (lyr. or older), F = Fawn (less than 1 yr.), M = Male, Fe = Female, Inoculum delivered by both I V and I M routes (except D35 — see text for details), DBS = Second sheep passage of bighorn sheep isolate.

** Dot . = Did not die, U = Undetermined, Parenthesis ( ) indicates degrees of peak temperature rise in F.

TABLE II. — Summary of virus isolation results from blood of deer experimentally exposed to BTV*

	Virus Isolations**								
	Deer								
Days P.I.	D1	D5	D7	D9	D2	D3	D4	D6	D8
0	.	.	.	—	.	—	—	—	—
1	.	.	—	.	.	.	.	—	.
2	.	.	.	—	.	.	.	+	.
3	+	—	—	.	+	+	+	+	.
4	+	+	.	+	+	+	+	+	.
5	+	+	+	.	+	+	+	+	.
6	+	+	+	.	+	+	X	+	.
7	+	+	.	—	+	+		+	+
8	+	+	.	X	+	+		+	—
9	+	.	X		X	X		X	X
10	+	.							
16	—	.							

*All deer exposed to BT-8, except D8 which was exposed to the Sonora 100 strain of BTV
**Dot . = Not tested, + = recovered, — = Virus not recovered, X = Death occured previous day

fied by centrifuging at 2,000 r.p.m. for 10 minutes. Using the method of Alexander (8), 0.3 ml. from each blood and tissue specimens was inoculated into the yolk-sac of each of six 7 day old embryonating chicken eggs. Inoculated eggs were incubated at 35°C the first 18-24 hours, and at 33.5°C the remainder of the incubation period.

The eggs were candled daily and the dead embryos were harvested. Blood samples were considered positive for BTV if by the third serial passage in eggs, the death rate had increased to at least five out of six and the embryos were hemorrhagic. All materials were routinely tested for bacterial contamination on blood agar and in thioglycollate broth. Selected samples of the embryo-lethal-agent were identified as BTV by virus-neutralization tests performed in suckling mice.

Adaptation to Mice. — Chicken-embryo-lethal-agents from D2 and D3 were adapted to suckling mice one to three days old following the method of Van den Ende, *et al* (4). Each of six to eight mice, constituting a group, received 0.02 ml. of a 10% chick embryo suspension by intracerebral inoculation. Each mouse received a second inoculation 2-5 hours after the first. Seven to eight days after exposure, or within 24 hours of the onset of illness, brains were harvested by group and a 10% suspension of brain was suspended in PBS. Two to three serial mouse passages of this material were required before death occurred regularly 7 to 10 days post inoculation. The mouse adapted isolates were identified by serum neutralization tests using pre-inoculation and convalescent serum samples of sheep (S63).

Virus neutralization tests in mice were performed on all of the deer and sheep serum samples collected. The constant serum-varying virus procedure was employed. The sera were heat inactivated at 56°C for 30 minutes. The serum-virus mixtures, containing antibiotics, were incubated for 30 minutes at room temperature and 30 minutes at 4°C as recommended by Kipps (9). Groups of six to eight suckling mice were individually inoculated intracerebrally with appropriate serum-virus mixtures. With each set of sera tested, a control litter was inoculated with BTV and either normal deer or normal lamb serum. The LD_{50} was calculated by the method of Reed and Muench (10).

Selected epizootic hemorrhagic disease (EHD) immune deer sera were also tested by this system.

Results

Signs and Lesions. — Experimental exposure to BTV resulted in clinical disease in all the deer and sheep (Table I). The infections were fatal for all of the fawns (D2, D3, D4, D6, D8, D35 and D36) and the adult female deer (D7), with death occurring from the fifth to eighth day post-inoculation. The sheep and the two male adult deer did not succumb.

Clinical signs observed in all fatal fawn cases, included a rise in temperature (Table I) that went as high as 106°F. A typical

TABLE III. — Summary of virus isolation results from tissues and body fluids of deer experimentally exposed to BT-8

	Virus Isolations*				
	Deer				
Tissue	D3	D4	D2	D36	D35
Spleen	+	+	+	+	+
Tongue	+	+	E	.	.
Liver	+	+	.	.	.
Kidney	+	+	.	.	.
Prescapular lymph node	.	+	.	.	.
Post mortem blood	+	+	.	.	.
Thoracic fluid	E	+	.	.	.
Urine	E	+	.	.	.

* + = Virus recovered, E = Equivocal results, Dot . = Not tested.

TABLE IV. Results of Mouse Neutralization Tests of Experimental Deer and Sheep Exposed to Bluetongue (BTV-8)

Serum source	LOG_{10} NEUTRALIZING INDEX* Pre-inoc. Sera	Conv. Sera**
Control Sheep	0	
S63	0	3.0
S1	0.23	3.0
S2	0	2.5
S3	0	3.0
Control Deer	0	
D1	0	3.0
D5	0.6	3.5
D2	0	
D3	0	1.35 (8)
D4	0.2	0.43 (4)
D6	0	2.5 (8)
D7	0	0.1 (6)

*Log_{10} neutralizing index calculated by subtracting the MLD_{50} titer of the virus in the presence of the test serum from that of the virus in the presence of the normal serum.
**Convalescent sera collected approximately 2 mos. p. i. unless indicated by days p. i. in parenthesis ( ) for animals that died. A dot, indicates serum not tested.

temperature response of deer is illustrated in Figure II. Anorexia usually began on the day of the peak in temperature and excessive salivation commonly occurred the day preceding death. All fawns became weak, lost their fear of man, and were recumbent one to two days prior to death. There was a clear, mucopurulent nasal discharge of varying degrees in all animals that succumbed. Crusts were formed in the nares of D2, D3, D4 and D8. A severe bloody diarrhea occurred in D6. Except for D7, all of the animals that died had swollen and cyanotic tongues. The only appreciable hematological change detected was a leucopenia. This was especially evident in deer that survived (Table IV).

The only sign of disease in the two adult male deer was a temperature rise of 4.6 and 2.2 degrees that occurred on the fifth and sixth day post inoculation and returned to normal in two to three days (Table I). Because of daily fluctuations it was not possible to evaluate the temperature response of the female deer (D7). On the day it succumbed, D7 was recumbent but attempted to rise. Her hind legs appeared paralyzed.

The clinical signs observed in the exposed sheep were a temperature rise on the sixth or seventh day post inoculation (Table I), anorexia at about the same time, a slight weight loss and some salivation.

Gross lesions in all deer that died included subendocardial hemorrhages, enteritis of the small and large intestine, and hemorrhages in the tongue. There were no tongue lesions in D7. The extent and severity of hemorrhaging varied between individuals, but was always present. The most severe hemorrhagic lesions occurred in D8. D36 had subcutaneous hemorrhages, an ulcer in the rumen approximately 1 cm. in diameter and peritonitis. There was a small hyperemic area of the buccal mucosa in D6 and extensive hyperemia of the buccal mucosa in D8. D7 had a necrotic fetus that was macerated to the stage of partial liquification.

Microscopically, either hyperemia, congestion or hemorrhages were usually seen in the heart, lungs, liver, spleen, kidneys, stomachs, intestines, adrenals, lymph nodes, thymus and tongue. A detailed description of the histopathology of experimental bluetongue in white-tailed deer was presented by Karstad and Trainer (11).

Virus Isolations. — Virus was detected in the blood of all the deer exposed to BTV (Table II). The viremia and temperature of D2 appeared to parallel one another and were considered typical of the temperature response and viremia (Figure II). Of the limited isolation attempts made from deer

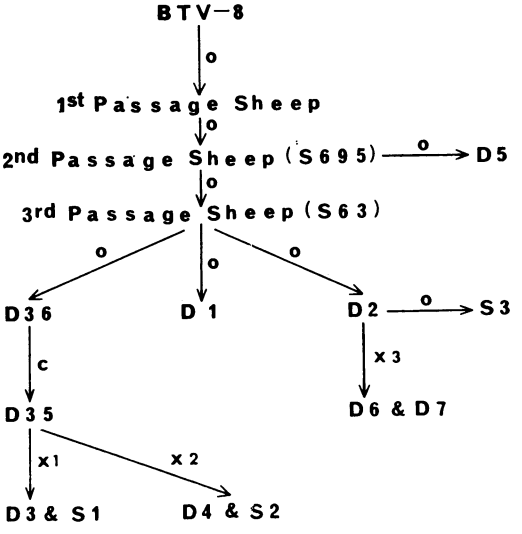

Fig. 1. Protocol of Experimental Exposure of Deer and Sheep to Bluetongue (BTV-8).*

* D = Deer, S = Sheep
O = Blood in O.C.G.
C = Contact (D35 not inoculated)
X1 = 10% spleen suspension (filtered)
X2 = 10% spleen suspension (not filtered & containing no antibiotics)
X3 = 10% spleen suspension (not filtered & containing antibiotics)

TABLE V. — Summary of Hematology Results of two BTV-8 Infected Deer, one which survived and one which succumbed.

Day of Inoculation	HEMATOLOGICAL VALUES* RBC (10^6) D1	RBC (10^6) D2	WBC (10^3) D1	WBC (10^3) D2	PCV D1	PCV D2	Sed. Rate (1 hr.) D1	Sed. Rate (1 hr.) D2
− 5	8.9	8.5	6.2	2.8	40	54	3.2	0.0
− 4	7.9	14.6	6.2	4.7	43	61	4.4	0.1
− 3	8.8	12.6	6.0	4.1	41	54	1.9	0.0
− 2	9.3	13.7	3.7	4.2	44	53	0.9	0.2
0	9.4	12.5	3.7	2.5	43	45	0.5	0.2
1	9.4	13.5	3.5	4.0	46	36	0.8	0.1
2	9.4	13.3	3.9	3.1	44	37	0.6	0.1
3	8.5	12.7	1.4	2.8	40	58	—	—
4	9.0	12.4	1.3	2.3	41	56	4.6	0.0
5	.	.	1.7	2.0	47	58	2.3	0.1
6	.	.	2.1	1.9	60	60.5	0.0	0.0
7	11.4	13.4	2.6	2.8	58	59.5	0.0	0.2
8	10.3	12.9	2.6	7.5	53	55.5	0.15	0.1
9	10.5	D	2.5	D	46	D	1.6	D
10	8.5		3.0		42		3.4	
11	.		.		—		.	
12	9.6	.	2.8		47		0.4	

*RBC = Red blood cells, WBC = White blood cells, PCV = Packed cell volume, Sed. = Sedimentation, Dot . = Not tested, D = Animal died.

tissues and fluids, BTV was detected in the spleen, tongue, liver kidney, lymph node, post mortem blood, thoracic fluid and urine (Table III).

Because of the requirement for adopting BTV to embryonating eggs by serial passage it was possible to quantitate BTV in the blood during the course of the infection or in the tissues and fluids at necropsy.

Neutralization Tests. — The convalescent serum of the third passage sheep (S63) was used as a standard and had a $\log_{10}$ neutralizing index of 3.0.

Convalescent sera from all of the animals neutralized the BTV from D2, and the results are summarized in Table IV. Convalescent sera from both surviving deer (D1 and D5) neutralized the virus, and of the deer that succumbed, the amount of neutralizing antibody appeared to depend on the length of survival time.

Convalescent serum from each of the experimental sheep (S1, S2 and S3) had a $\log_{10}$ neutralizing index of 2.5 or 3.0.

To test for a possible serologic relationship between bluetongue and epizootic hemorrhagic disease of deer, three EHD immune deer sera were incorporated into the same test system used for the BTV deer sera. No significant neutralization was shown by the EHD sera.

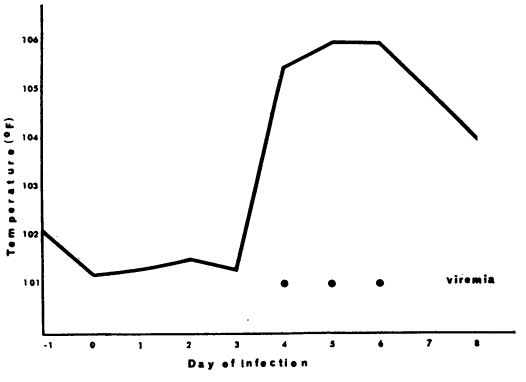

Fig. 2. A typical temperature and viremia response of deer to experimental bluetongue, as observed in D2.

Discussion

These limited studies show that experimental bluetongue can produce both fatal and non-fatal infections in deer. It appears that deer less than a year of age may be more susceptible to fatal infections since all seven exposed fawns succumbed. The results of the exposure of the fawn D8 to the bighorn sheep BTV isolate were similar to those observed in the fawns exposed to BTV-8. The results with the adult pregnant doe (D7) are difficult to interpret. This deer was extremely wild and it was necessary to immobilize her with succinycholine chloride to collect specimens. It is possible that mechanical injury contributed to her recumbency and death. Also, at necroposy

a necrotic fetus was found, and its role in her death was not determined. It was evident from the degree of maceration that the death of the fetus occurred prior to BTV exposure.

The two adult male deer which survived BTV challenge were immune to EHD. It is not known what effect this may have had on the BTV responses in these deer. As a result, the specific effect of age on susceptibility was not fully established. It was noted that the incubation period, signs and gross lesions observed for bluetongue in deer were similar to those described for EHD (11, 12). Further studies are necessary to elucidate the possible decreased virulence for adults as well as any relationship between EHD and the various antigenic types of bluetongue.

Many of the signs and lesions observed for bluetongue of deer were typical of those described for sheep, however, some of the classical lesions described for this disease in sheep were not seen. These included the hoof lesions and the excoriations of the nasal and oral mucosa. Perhaps an explanation for the lack of these lesions in the fawns was the peracute to acute course of the disease.

The apparent contact transmission of bluetongue from D36 to D35 was unexpected and unexplained. By necessity, the autopsy of D36 was performed in the isolation unit occupied by D35 (a contact control). Since the animals were housed on concrete and could have incurred abrasions on the legs, or other areas, the exposure of D35 could have occurred by contamination of skin abrasions.

A viremia occurred in experimental bluetongue of deer and virus was detected in the blood for as long as ten days after exposure. BTV was also detected in a variety of deer tissues and urine. Of the tissues studied, the spleen appeared to be the best source of virus.

These preliminary studies indicate that deer are susceptible to BTV and might play a role in its epizootiology. Recently a "seminatural" outbreak of bluetongue occurred in a herd of captive deer in Texas (13), illustrating that BTV in deer is probably not confined to experimental exposures. Although the status of bluetongue in deer is still unknown, it might, as with cattle, serve as a source of virus for the insect vector during epizootics of the disease. Deer may serve as a reservoir during interepizootic periods. The need for further investigations to clarify the significance and role of deer in the natural history of bluetongue is apparent.

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