BLUETONGUE VIRUS AND WHITE-TAILED DEER IN AN ENZOOTIC AREA OF TEXAS 1

Author: HOFF, GERALD L.

Source: Journal of Wildlife Diseases, 10(2) : 158-163

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-10.2.158

BLUETONGUE VIRUS AND WHITE-TAILED DEER IN AN ENZOOTIC AREA OF TEXAS[1]

GERALD L. HOFF, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin, 53706, U.S.A.[2]

DANIEL O. TRAINER, Dean, College of Natural Resources, University of Wisconsin, Stevens Point, Wisconsin, 54481, U.S.A.

MICHAEL M. JOCHIM, Animal Disease Research Laboratory, U.S. Department of Agriculture, Denver, Colorado, 80225, U.S.A.

Abstract: A ten-year serologic and virologic investigation into the activity of enzootic bluetongue (BT) virus was conducted in southern Texas white-tailed deer (*Odocoileus virginianus texanus*). Eighty-nine percent of 484 adult deer, 36% of 129 juvenile deer and 93% of 182 neonatal deer were sero-positive of BT. Antibody was not detected in fetal fawns but was found in colostrum samples. Sentinel fawn studies demonstrated that maternal antibody persists at least 8 weeks and that BT was transmitted during the fall months. The virus was isolated from a sentinel fawn but could not be recovered from deer with antibody or with organizing lesions suggestive of previous BT infection. Virus was not isolated from deer ectoparasites.

INTRODUCTION

Many facets concerning the epizootiology of bluetongue (BT) are incompletely understood, especially those involving wildlife. Several wild ruminants have been shown to be experimentally susceptible to BT virus including the blesbok (*Damaliscus albifrons*),[8] the white-tailed deer (*Odocoileus virginianus*),[12] the pronghorn antelope (*Antilocapra americana*)[3] and the American elk (*Cervus canadensis*).[7] In addition, naturally occurring cases of BT have occurred in bighorn sheep (*Ovis canadensis*),[9] white-tailed deer,[11] muntjac (*Muntiacus reevesi*) and greater kudu (*Tragalaphus capensis*).[4]

Results of a serologic survey of North American wild ruminants[14] suggested that BT occurred in a variety of wildlife and was enzootic in a deer population at the Welder Wildlife Refuge in southern Texas; however, the importance of wildlife in the natural history of BT remained unknown. To add to our knowledge of the epizootiology of BT in a wild population, a serologic and virologic investigation of deer at the Welder Wildlife Refuge was undertaken.

MATERIALS AND METHODS

Study area:

The Welder Wildlife Refuge consists of 31.7 km^2 of cattle rangeland along the coastal bend of south Texas in San Patricio county. Throughout the study, the deer population remained relatively stable, averaging approximately 48 deer/km^2, or 125 deer per square mile. Cattle ranching operations were also conducted on the Refuge during this period.

[1] Study supported in part by funds provided by the Welder Wildlife Foundation, Sinton, Texas, 78387. Welder Wildlife Contribution No. 157.

[2] Hoff present address: Veterinary Public Health Section, Florida Division of Health, P.O. Box 210, Jacksonville, Florida, 32201 U.S.A.

Free-ranging deer:

Between 1963 and 1972, deer on the Refuge were periodically collected by trapping or shooting. The deer were divided in to age cohorts as follows. neonatal fawns consisted of animals 2 weeks or less of age; juveniles were 2 weeks to 15 months of age; and adults were 15 months and older.

A blood sample was obtained from the jugular vein of each deer. Prior to 1969, the blood sample was used only for serum. Between 1969 and 1972, additional aliquots of blood were mixed 1:1 in a solution of oxalate-phenol-glycerine[3] (OPG) for virus isolation attempts.

All deer which were shot were necropsied and examined grossly for lesions suggestive of BT virus infection. From 1969 to 1972, ectoparasites and selected tissue and fluid samples were collected from each deer and from fetal fawns. The samples were placed in equal or greater volumes of OPG and held at 4 C until assayed for virus.

Sentinel fawns:

During the summers of 1969, 1970, and 1971 sentinel fawns were used to monitor BT virus activity. In both 1969 and 1970, the fawns were brought to the Refuge from the University of Wisconsin experimental deer herd under permit from the Texas Parks and Wildlife Department. In 1971, the fawns were captured on the Refuge shortly after being born to free-ranging does.

The fawns from Wisconsin were bled for baseline blood subsequent to their arrival at the Refuge. The Texas fawns were bled at the time of capture. In all cases, the fawns were maintained at selected sites on the Refuge and bled at periodic intervals for serology and virus isolation. At the end of each summer, some of the sentinel fawns were killed. The remaining animals were allowed to serve as sentinels through the fall months, although they were not bled again until the following January or February unless they became overtly ill.

Serology and virus isolation attempts:

All sera were heat-inactivated at 56 C for 30 minutes prior to testing. Throughout the study, BT virus strain BT-8 was used as the test antigen. The group-specific agar gel precipitin test (AGP)[5] was used to test sera collected from 1963 to 1969. Sera collected in 1970, 1971, and 1972 were tested for BT neutralizing antibodies using the L-cell virus plaque reduction neutralization test (PRN)[13]

Virus isolation attempts were conducted on monolayer cultures of L-cells.[4] Fluid samples in OPG were diluted 1:10 in Hank's balanced salt solution prior to inoculation of the cultures. The OPG was drained off the tissue and ectoparasite samples, which were then ground with TenBroeck tissue grinders and the resulting homogenates resuspended (10% wt/vol) for inoculation. Individual 0.1 ml portions of all the samples were inoculated onto dried monolayer cultures and for 7 days the cultures were observed for development of cytopathic effect. Virus isolates were identified by neutralization tests with reference antisera and viruses.

RESULTS

Free-ranging deer:

Seventy-four fetal and 795 adult, juvenile and neonatal deer were examined serologically, pathologically or virologically for evidence of past or current BT virus infection. Of 484 adult deer, 431 or 89% were sero-positive for BT, while 93% (170/182) of the neonatal fawns and 36% (47/29) of the juveniles were reactors (Table 1). Antibody was not detected in ten fetal fawns tested, despite the fact that the mothers of four possessed antibody. These fetuses were collected shortly before they would have

[3] OPG—Potassium oxalate, 5 gm; phenol, 5 gm; glycerol, 500 ml; double distilled water, 500 ml.

been born. Although the composition and sample size varied from year to year, there was no substantial difference in the number of reactors annually, especially in the 1967 and 1969 samples involving only small numbers of juvenile animals are omitted.

There was no significant difference between total reactor rates of males and females (Table 2). However, among the juvenile portion of the population, males had a significantly higher reactor rate (Chi square, P.01) than females, 49% and 20% respectively.

When the fawns from different years were grouped according to specific age, antibody occurred in 95% of the neonatal fawns, dropped to an average of 24% in the 3-9 month animals and increased to 50% in the 1 year-old deer

TABLE 1. A summary of BT virus serologic results of white-tailed deer from the Welder Wildlife Refuge, 1963 to 1972.

Year	Serologic Results [1]							
	Adults		Juveniles		Neonatal		Total	
1963	41/44	(93)	3/4	(75)	39/40	(97)	83/88	(94)
1964	58/62	(93)	13/19	(68)	30/33	(91)	101/114	(88)
1965	106/118	(90)	9/32	(28)	78/80	(97)	193/230	(84)
1966	76/78	(97)	0/2	(0)	—		76/80	(95)
1967	—		3/16	(19)	—		3/16	(19)
1968	42/55	(76)	3/14	(21)	—		45/69	(65)
1969	—		4/18	(22)	—		4/18	(22)
1970	43/47	(92)	10/13	(77)	8/11	(70)	61/71	(86)
1971	44/55	(80)	0/6	(0)	15/18	(83)	59/79	(75)
1972	21/25	(84)	2/5	(40)	—		23/30	(77)
Total	431/484	(89)	47/129	(36)	170/182	(93)	648/795	(82)

[1] Results are recorded as the number of positive reactors (numerator) over the number of sera tested (denominator) and the percent of reactors is in parenthesis ( ). Sera from 1963 to 1969 tested in AGP test, while 1970 to 1972 tested in PRN test.

TABLE 2. A summary of BT virus serologic results of male and female deer from the Welder Wildlife Refuge, 1963-1972.

Age	Serologic Results [1]					
	Male		Female		Total	
Adult	188/208	(90)	243/276	(88)	431/484	(89)
Juvenile	36/73	(49)	11/56	(20)	47/129	(36)
Neonatal	82/89	(92)	88/93	(95)	170/182	(93)
Total	306/370	(83)	342/425	(80)	648/795	(82)

[1] Results are recorded as the number of positive reactors over the number of sera tested. The percent of reactors is in parenthesis ( ). Sera from 1963 to 1969 tested in AGP test, while 1970 to 1972 tested in PRN test.

(Table 3). The high reactor rate in neonatal fawns was determined to be the result of transfer of maternal antibodies in colostrum, since BT neutralization titers of up to 1:10,000 were observed in samples from BT sero-positive does. Antibody was not detected in the milk of lactating does.

During the study, only two juvenile does and one fetus had lesions suggestive of a hemorrhagic disease. The juvenile does were 6 and 7 months of age and were collected in two different years. On gross examination, both deer had extensive ecchymotic, subserosal hemorrhages on the diaphragm, large and small intestines, and stomach. Hemorrhages were also present at the base of the pulmonary artery, aorta and endocardium of the left ventricle. Petechial hemorrhages were observed on the inner surface of the trachea. In one deer, the larynx was hemorrhagic and edematous; suffusions were present on the hind legs and back. The tongues of both deer were cyanotic.

Histopathological examination revealed that the hemorrhages were organizing; hemosiderin was common in all tissues examined. Edema was observed in the heart, liver, skeletal masculature, kidney and tongue.

The fetus was examined only grossly and was hemorrhagic and necrotic. The crown-rump length of the fetus was 67 mm indicating that it was approximately 59 days old.[10]

Virus was not recovered from either of the does or from the fetus. One of the does possessed BT nutralizing antibody while the other did not.

No isolations of BT were made from 1,817 tissue, blood and other fluid samples collected from 195 deer and 74 fetuses. Also, virus was not isolated from 565 ticks, *Amblyomma* ssp. and *Ixodes* spp., or 1,700 keds, *Liptopterna mazamae*, collected from the deer.

Sentinel fawns:

During the summers of 1969 and 1970, none of the sentinel fawns from Wisconsin serologically converted for BT, nor was virus isolated from the blood of any of these deer. In 1971, nine of the ten fawns acquired on the Refuge possessed maternal neutralizing antibody to BT at the time of capture. However, only four of these animals were successfully reared and their maternal antibodies persisted for more than 8 weeks (Table 4). Virus was not recovered from the fawns during the summer of 1971.

In both 1969 and 1970, two fawns were allowed to serve as sentinels during the fall months. In 1971, this number was increased to four animals. On November 12, 1969 and on October 29, 1971, sentinel fawns died of a clinical disease characterized by depression and severe respiratory distress. Gross and histological lesions resembled those associated with experimental BT in white-tailed deer.[6] BT virus was recovered from the blood and various organs of the 1969 case, and was shown to be indistinguishable from BT-8. Regrettably, tissues for

TABLE 3. Summary of BT serologic results from different aged fawns from the Welder Wildlife Refuge (1963-1972).

Age (months)	No. Tested	No. Reactors	Percent Reactors
1	173	164	95
3	29	5	17
6	25	9	36
9	17	3	18
12	16	8	50

TABLE 4. Persistence of maternal antibody to BT virus in four white-tailed deer fawns from the Welder Wildlife Refuge.

Fawn No.	Week of Month					
	June		July		August	
	2	4	2	4	2	4
1	160[1]	320	160	40	80	NT[2]
2[3]	2560	1280	1280	640	640	NT
3	40	40	Neg	Neg	Neg	NT
4	640	1280	320	640	40	NT

[1] Titers expressed are reciprocals of highest serum dilution which caused a 50% or greater reduction in PRN test.

[2] NT — not tested.

[3] This fawn subsequently died during the 4th week of October of a disease highly suggestive of BT. At the time of death, the deer had a 1:640 antibody titer to BT. The other three fawns had no detectable BT titer when bled in following February.

virus isolation from the 1971 fawn were accidentally destroyed before they could be assayed.

Serological evidence of BT virus transmission was detected in 1969 and 1970 with sero-conversion of the remaining sentinel fawns. In January 1970, the surviving sentinel fawn from the previous summer was killed and organizing lesions suggestive of BT infection were observed; virus was not isolated. None of the three surviving fawns from 1971 converted serologically for BT.

DISCUSSION

Of 795 deer tested, 82% were seropositive for BT, suggesting that the Welder Wildlife Refuge is an enzootic area for BT. The serologic conversion of sentinel deer and the isolation of BT from a fawn in 1969, supports the validity of these findings. Additionally, BT antibodies were detected in more than 88% of the cattle on the Refuge.[2] Between 1970 and 1972, neutralizing antibodies to the closely related virus of EHD were not detected in the cattle or deer.[2]

Experimentally, BT in deer can result in an acute, rapidly fatal disease or in a subclinical infection with persistent viremia.[12] The serologic data suggests that the majority of the deer infected on the Refuge experienced the subclinical or mild form of the disease. The acute form was manifested in the deaths of sentinel fawns in 1969 and possibly in 1971. These observations could be due to different BT virus strains, intensity or route of exposure, or timing of exposure.

Analysis of BT serology by age cohorts, shows a decrease in antibody prevalence from birth to 12 months of age, followed by an increase to high levels by the second year of life and thereafter. Based on sentinel fawns and combinations of does and their fawns or fetuses, the high seropositivity (93%) in neonatal fawns can be attributed to maternal antibody derived from the colostrum. As this antibody deteriorates, so does the reactor rate in juveniles to about 24%. All fawns are born in June and the maternal antibody, as illustrated by the 1971 sentinel deer, persists at least 8 weeks. The present study and other reports[11] show that in Texas BT exposure often occurs in early fall. At this time sufficient antibody may still exist in fawns to provide protection against fatal BT.

That 49% of male versus 20% of the female juvenile deer were positive is of interest, especially since there is a selective mortality occurring among male

juveniles on the Refuge at a 2:1 ratio. An explanation for this possible differential exposure in unavailable at this time.

It cannot be proven that the hemorrhagic and necrotic fetus found in 1971 was the result of BT infection of the doe. Experimentally induced BT during the first trimester of gestation in white-tailed deer has been shown to have adverse effects on fetal development.[13] Since the 7 month gestation period in white-tailed deer in the part of southern Texas where the Refuge is located, begins in November-December,[1] it is conceivable that BT could have caused the death of this fetus.

The value of a continued epizootiological investigation of this enzootic area to establish the natural history of BT in southern Texas is obvious. The needed integration of entomological and ecological studies with the virological and serological investigation is also evident.

Acknowledgements

Appreciation is expressed to Dr. J. O. Murray and to Mr. Caleb Glazener of the Welder Wildlife Foundation for their contributions to the various aspects of this study and to Dr. Lars Karstad of the University of Guelph for histopathology.

LITERATURE CITED

1. DAVIS, W. B. 1966. The Mammals of Texas. Texas Parks & Wildlife Dept., Austin, Texas. Bulletin No. 41.
2. HOFF, G. L. 1972. Epizootiological studies on the viruses of bluetongue and epizootic hemorrhagic disease in wild ruminants. Ph.D. Thesis. Univ. Wisconsin, Madison, Wisconsin. 159 pp.
3. HOFF, G. L. and D. O. TRAINER. 1972. Bluetongue virus in pronghorn antelope. Amer. J. Vet. Res. 33: 1013-1016.
4. HOFF, G. L., L. A. GRINER and D. O. TRAINER. 1973. Bluetongue virus in exotic ruminants. J. Amer. Vet. Med. Assoc. 163: 565-567.
5. JOCHIM, M. M. and T. L. CHOW. 1969. Immunodiffusion of bluetongue virus. Amer. J. Vet. Res. 30: 33-41.
6. KARSTAD, L. and D. O. TRAINER. 1967. Histopathology of experimental bluetongue disease of white-tailed deer. Can. Vet. J. 8: 247-254.
7. MURRAY, J. O. and D. O. TRAINER. 1970. Bluetongue virus in North American elk. J. Wildl. Dis. 6: 144-148.
8. NEITZ, W. O. 1933. The blesbok (*Damaliscus albifrons*) as a carrier of heartwater and bluetongue. J. S. Afr. Vet. Med. Assoc. 4: 24-26.
9. ROBINSON, R. M., T. L. HAILEY, C. W. LIVINGSTON and J. W. THOMAS. 1967. Bluetongue in the desert bighorn sheep. J. Wildl. Mgmt. 31: 165-168.
10. SHORT, C. 1970. Morphological development and aging of mule and white-tailed deer fetuses. J. Wildl. Mgmt. 34: 383-388.
11. STAIR, E. L., R. M. ROBINSON and L. P. JONES. 1968. Spontaneous bluetongue in Texas white-tailed deer. Pathol. Vet. 5: 164-173.
12. THOMAS, F. C. and D. O. TRAINER. 1970. Bluetongue virus in white-tailed deer. Amer. J. Vet. Res. 31: 271-278.
13. THOMAS, F. C. and D. O. TRAINER. 1970. Bluetongue virus (1) In pregnant white-tailed deer (2) A plaque reduction neutralization test. J. Wildl. Dis. 6: 384-388.
14. TRAINER, D. O. and M. M. JOCHIM. 1969. Serologic evidence of bluetongue in wild ruminants of North America. Amer. J. Vet. Res. 30: 2007-2011.

Received for publication 14 December 1973