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SHORT COMMUNICATIONS

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Antibodies to Bluetongue and Epizootic Hemorrhagic Disease Viruses in a Barrier Island White-tailed Deer Population

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ABSTRACT: From 1981 through 1989, serum samples from 855 white-tailed deer (*Odocoileus virginianus*) from Ossabaw Island, Georgia (USA), were tested for antibodies to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV). During this period, prevalence of precipitating antibodies to BTV and EHDV as determined by agar gel immunodiffusion (AGID) tests decreased from 74% to 3% and from 34% to 1%, respectively. Antibodies were detected in serum samples from 0.5-yr-old deer only during 1981, 1982, and 1983, and with few exceptions, positive serological results after 1983 were restricted to older age classes. A decrease in prevalence of precipitating antibodies to BTV and EHDV in age classes exposed during 1981 indicates that AGID results from white-tailed deer populations underestimate the extent of previous exposure to these viruses. Serum neutralization test results from AGID-positive deer indicated that BTV 11 was the principal serotype responsible for infections during 1981. Since 1983, this serotype has been replaced by BTV 13; however, there has been a low level of transmission within the herd. Infection with EHDV 2 appeared most prevalent during 1982; as with BTV 13, there has been limited transmission in this high density deer population since 1983.

Key words: Bluetongue virus, epizootic hemorrhagic disease virus, hemorrhagic disease, white-tailed deer, *Odocoileus virginianus*, antibodies, serology.

Although precipitating antibodies to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) (Reoviridae: Orbivirus) are consistently detected in white-tailed deer (*Odocoileus virginianus*) in the southeastern United States (Couvillion et al., 1981), patterns of infection vary considerably between years, latitude and physiographical provinces (Stallknecht et al., 1991). On the barrier islands of Georgia, BTV and EHDV infection of white-tailed deer occurs sporadically (Stallknecht et al., 1991), and, as in other areas, infections may be inapparent (Kocan et al., 1982) or may result in a clinical syndrome referred to as hemorrhagic disease (HD) (Thomas et al., 1974).

In a recent serological survey of Georgia white-tailed deer from 1981 through 1988, we reported that prevalence of antibodies to BTV and EHDV in deer from Ossabaw Island, Georgia, (31°47′N, 81°07′W) decreased from 74% to 3% and from 34% to 1%, respectively (Stallknecht et al., 1991). Deer mortality attributable to HD was observed on Ossabaw Island during the summer of 1980 (Southeastern Cooperative Wildlife Disease Study, unpubl. data). Although virus was not isolated, necropsy findings, the pattern of mortality, and the time of year were suggestive of HD.

Since much about the epizootiology of these viruses in white-tailed deer populations remains undefined, especially with regard to mechanisms of viral persistence, we conducted a retrospective serological evaluation of BTV and EHDV in this isolated population. The objectives of this study were to determine (1) annual patterns of BTV and EHDV infection, (2) BTV and EHDV serotypes present in this population and (3) the extent of persistence of precipitating antibodies to both BTV and

EHDV in naturally infected white-tailed deer.

Serum samples were collected from hunter-killed white-tailed deer on Ossabaw Island, Georgia, from October through December from 1981 through 1989. Deer ages were estimated by tooth eruption and wear patterns (Severinghaus, 1949). Ossabaw Island (31°47′N, 81°07′W) is a heritage preserve administered by the Georgia Department of Natural Resources and includes 10,117 ha consisting of 4,775 ha of upland habitats and 5,342 ha of saltwater, brackish, and freshwater marshes (Fletcher et al., 1985). Cattle are present on the Island, and bovine sera collected during 1981 as part of surveillance for vesicular stomatitis virus (Fletcher et al., 1985) were retrospectively tested for BTV and EHDV antibodies during the present study.

Sera were tested for BTV and EHDV antibodies by agar gel immunodiffusion (AGID) (Pearson and Jochim, 1979) at the Georgia Diagnostic Assistance Laboratory (College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA) or at the National Veterinary Services Laboratories (NVSL; Science and Technology, Animal and Plant Health Inspection Service, USDA, Ames, Iowa 50010, USA). All sera were stored at −20C prior to further testing.

During 1990, a retrospective study was made, whereby available sera which tested positive on BTV or EHDV AGID tests were heat inactivated (55 C for 30 min) and tested by serum neutralization (SN) test. All serum dilutions, viral dilutions, and cell suspensions were made in a maintenance medium consisting of Dulbecco's modified Eagle's medium supplemented with 3% heat inactivated calf serum and antibiotics (100 units penicillin, 0.1 mg streptomycin, and 0.25 μg amphotericin B/ml). For initial screening, 50 μl of BTV 2, 10, 11, 13, and 17 and EHDV 1 and 2 at 200 to 600 median tissue culture infective doses ($TCID_{50}$)/50 μl, were added to paired wells containing 50 μl of a 1:5 serum dilution. Following incubation for 1 hr at 37 C, 150 μl of maintenance medium containing approximately 2.5×10^4 baby hamster kidney (BHK_{21}) cells/ml was added to each well. Plates were incubated at 37 C in a 5% CO_2 atmosphere and were read at 48 to 72 hr. Serum dilutions in wells exhibiting >25% cytopathic effect were considered negative. All sera testing positive at a 1:10 dilution were further tested against the respective BTV and EHDV serotypes at two-fold dilutions ranging from 1:10 to 1:320.

Serological evidence considered indicative of previous exposure to a specific BTV or EHDV serotype consisted of either a monospecific test result at a ≥1:20 serum dilution or greater or the presence of clusters of seropositive results to a specific serotype within a given year (Taylor et al., 1985). Prevalence of precipitating antibodies to BTV and EHDV were tested by age class and year using a G-test for independence (Sokal and Rohlf, 1981).

Prevalence of precipitating antibodies to BTV (Table 1) and EHDV (Table 2) in 855 white-tailed deer was dependent on both year and age class. A decrease in antibody prevalence from 1981 to 1989 was observed with BTV ($P < 0.001$) and EHDV ($P < 0.001$). Antibody prevalence increased with age for both BTV ($P < 0.001$) and EHDV ($P < 0.001$).

Precipitating antibodies to BTV and/or EHDV were detected in 0.5-yr-old deer only in 1981, 1982, and 1983. Only 5 of 333 (1%) sampled deer from age classes born after 1983 were seropositive to either of these viruses.

Observed prevalence of antibodies to BTV and EHDV from 1981 through 1986 in age classes exposed during 1981 are shown in Figure 1. Antibody prevalence to BTV and EHDV in these age classes decreased each year from 1981 and 1982, respectively. However, seropositive animals in these age classes were detected throughout these years.

Sera from 70 AGID positive deer (BTV or EHDV) were tested by serum neutral-

TABLE 1. Prevalence of precipitating antibodies to bluetongue virus in white-tailed deer by age class, Ossabaw Island, Georgia, 1981 to 1989.

Age class (yr)	Year				
	1981	1982	1983	1984	1985
0.5	10/12 (83%)*	3/20 (15%)	2/24 (8%)	0/16 (0%)	0/13 (0%)
1.5	10/12 (83%)	3/12 (25%)	2/30 (7%)	2/26 (8%)	1/28 (4%)
2.5	6/6 (100%)	8/12 (67%)	3/8 (37%)	2/29 (7%)	1/23 (4%)
3.5	10/15 (75%)	11/21 (52%)	4/8 (50%)	3/12 (25%)	0/22 (0%)
4.5	5/10 (50%)	6/14 (43%)	6/10 (60%)	1/8 (12%)	0/7 (0%)
5.5	4/6 (67%)	7/11 (64%)	7/9 (78%)	3/5 (60%)	0/3 (0%)
≥6.5	3/4 (75%)	8/11 (73%)	3/7 (43%)	3/12 (25%)	4/13 (31%)
Total	48/65 (74%)	46/101 (46%)	27/96 (28%)	14/108 (13%)	6/109 (5%)

* Number seropositive/number tested (% seropositive).

ization (Table 3). Sera were available for all years except 1982. Serum neutralizing antibodies at a ≥1:20 serum dilution or greater were detected against BTV 11, BTV 13, and EHDV 2. Monospecific serum neutralizing antibodies to BTV 11 were detected in 1981, 1983, 1984, 1985, and 1988. Clusters of deer seropositive to BTV 11 were detected from 1981 through 1984. Mean log antibody titer to BTV 11 decreased from 1.74 (n = 24) to 1.38 (n = 19) to 1.17 (n = 7) from 1981 to 1983 to 1984, respectively. All positive serologic results to BTV 11 from 1984 to 1989 were restricted to age classes which were represented during the period 1981 to 1983.

Serum neutralizing antibodies to BTV 13 were detected from deer sampled in 1984, 1985, 1988, and 1989. However, monospecific serologic results were observed only in 1984 and 1989. These included a 2.5-yr-old deer sampled in 1989. Due to low overall antibody prevalence to BTV 13 in this population, no clusters of seropositive results to this virus were detected.

Monospecific antibodies to EHDV 2 were detected in deer from all tested years except 1984. As with BTV 13, antibodies to EHDV 2 were detected in age-classes which were not present during 1981 through 1983.

Precipitating antibodies to BTV and EHDV were detected in 10 (13%) and 5 (7%) of 75 cattle sampled in 1981, respectively. Three sera were tested by serum neutralization. Monospecific antibodies to EHDV 2 were detected in three cows, and antibodies to BTV 11 were detected in one.

Although HD-related mortality has not been observed in this white-tailed deer population since 1980, results from the 0.5-

TABLE 2. Prevalence of precipitating antibodies to epizootic hemorrhagic disease virus in white-tailed deer by age class, Ossabaw Island, Georgia, 1981 to 1989.

Age class (yr)	Year				
	1981	1982	1983	1984	1985
0.5	6/12 (50%)*	2/20 (10%)	1/24 (4%)	0/16 (0%)	0/13 (0%)
1.5	2/12 (17%)	4/12 (33%)	1/30 (3%)	1/26 (4%)	0/28 (0%)
2.5	3/6 (50%)	5/12 (42%)	2/8 (25%)	1/29 (3%)	0/23 (0%)
3.5	4/15 (27%)	10/21 (48%)	1/8 (12%)	2/12 (17%)	0/22 (0%)
4.5	2/10 (20%)	8/14 (57%)	2/10 (20%)	0/8 (0%)	0/7 (0%)
5.5	4/6 (67%)	5/11 (45%)	5/9 (56%)	3/5 (60%)	0/3 (0%)
≥6.5	1/4 (25%)	5/11 (45%)	4/7 (57%)	3/12 (25%)	4/13 (31%)
Total	22/65 (34%)	39/101 (39%)	16/96 (17%)	10/108 (9%)	4/109 (4%)

* Number seropositive/number tested (% seropositive).

TABLE 1. Continued.

Year				
1986	1987	1988	1989	Total
0/8 (0%)	0/11 (0%)	0/21 (0%)	0/17 (0%)	15/142 (11%)
0/14 (0%)	0/11 (0%)	0/18 (0%)	0/24 (0%)	18/175 (10%)
0/23 (0%)	0/15 (0%)	0/19 (0%)	1/23 (4%)	21/158 (13%)
0/26 (0%)	0/15 (0%)	1/18 (6%)	1/21 (5%)	30/158 (19%)
0/5 (0%)	0/15 (0%)	0/4 (0%)	0/10 (0%)	18/83 (22%)
0/5 (0%)	0/3 (0%)	1/14 (7%)	0/4 (0%)	22/60 (37%)
0/7 (0%)	1/6 (17%)	1/12 (8%)	1/7 (14%)	24/79 (30%)
0/88 (0%)	1/76 (1%)	3/106 (3%)	3/106 (3%)	148/855 (17%)

yr and 1.5-yr age classes suggest that BTV 11 and EHDV 2 transmission occurred from 1981 through 1983. Although both viruses appear to have been present during that time, BTV 11 was most prevalent, with serum neutralizing antibodies to this virus present in 100% of deer tested by SN from 1981. In a statewide survey of cattle and deer in 1980, Odiawa et al. (1985) reported that BTV 11, BTV 17, EHDV 2, and EHDV 1 were present in Georgia.

Since 1983, there has been a very low level of detectable BTV or EHDV transmission on Ossabaw Island, with most seropositive animals representing older age-classes which were alive during the period 1980 to 1983. With the exception of a single observation of hoof lesions in a 2.5-yr-old deer from 1988 which also had monospecific serum neutralizing antibodies to BTV 13, no acute or chronic HD has been observed in this herd since 1980.

Over the surveillance period, there has been no indication of BTV 11 infection after 1981 to 1983. All infections after 1983 appear to have involved BTV 13 or EHDV 2. Serotype shifts in BTV have been previously reported (Stott et al., 1981; Homan et al., 1990) but cannot be explained in our study area at this time.

The decline in overall infection since 1981 partially may relate to a decrease in viral transmission from host to vector populations. A reduction in availability of viremic hosts may have resulted from increased herd immunity associated with the 1981 outbreak. However, while host related factors may explain the disappearance of BTV 11 from this population they do not adequately explain the restricted transmission of BTV 13 and EHDV 2 in this high density population (one deer/4 ha) (Georgia Department of Natural Resources, unpubl. data).

TABLE 2. Continued.

Year				
1986	1987	1988	1989	Total
0/8 (0%)	0/11 (0%)	0/21 (0%)	0/17 (0%)	9/142 (6%)
0/14 (0%)	0/11 (0%)	0/18 (0%)	0/24 (0%)	8/175 (5%)
0/23 (0%)	0/15 (0%)	0/19 (0%)	0/23 (0%)	11/158 (7%)
1/26 (4%)	0/15 (0%)	1/18 (6%)	1/21 (5%)	20/158 (13%)
0/5 (0%)	3/15 (20%)	0/4 (0%)	0/10 (0%)	15/83 (18%)
1/5 (20%)	0/3 (0%)	1/14 (7%)	0/4 (0%)	19/60 (32%)
1/7 (14%)	2/6 (33%)	0/12 (0%)	0/7 (0%)	20/79 (25%)
3/88 (3%)	5/76 (7%)	2/106 (2%)	1/106 (1%)	103/855 (13%)

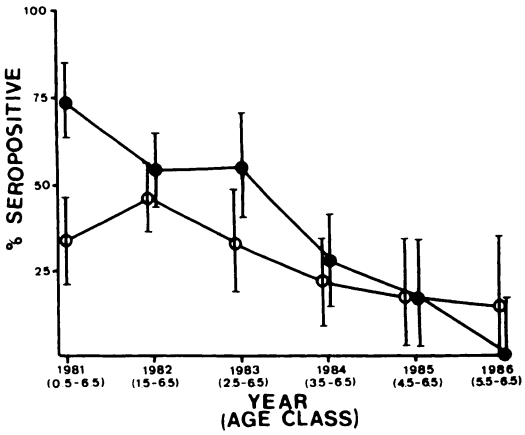

FIGURE 1. Reduction in prevalence of precipitating antibodies to bluetongue virus ●——● and epizootic hemorrhagic disease virus O——O in white-tailed deer age-classes present and potentially exposed during 1981, Ossabaw Island, Georgia.

Since there is little or no herd immunity at this time, restricted transmission of BTV 13 and EHDV 2 in this population is most likely related to vector species composition or population dynamics. Although *Culicoides* spp. are present on Ossabaw Island, *C. furens* is the only species which has been identified (Stallknecht et al., 1987). The status of *C. variipennis*, which represents the principal vector of BTV and EHDV in the United States (Mullen et al., 1985), on Ossabaw and other barrier islands of Georgia is unknown. In a survey of *Culicoides* spp. in coastal Georgia, Hagan and Wirth (1985) reported *C. variipennis* from mainland Chatham County, Georgia, in which Ossabaw Island is located. Collections on Sea Island, Georgia, a barrier island located approximately 66 km south-

TABLE 3. Serum neutralizing antibodies to bluetongue and epizootic hemorrhagic disease viruses in white-tailed deer, Ossabaw Island, Georgia, 1981 to 1989.

Year	Number tested	Serotype	Number positive	Number monospecific[a]	Age range[b] (yr)	Antibody titer range
1981	24	EHDV 2[c]	3	3 (3)	1.5–5.5	20–80
		BTV 11	24	24 (23)	0.5–6.5+	10–160
1983	19	EHDV 1	3	1	1.5	10
		EHDV 2	9	9 (5)	3.5–6.5+	10–320
		BTV 11	19	19 (12)	1.5–6.5+	10–320
1984	11	EHDV 1	2	2	1.5–3.5	10
		EHDV 2	2	2	5.5–6.5+	10
		BTV 10	1	0	5.5	10
		BTV 11	7	6 (3)	3.5–6.5+	10–160
		BTV 13	3	2 (1)	1.5–5.5	10–80
		BTV 17	1	0	5.5	10
1985	4	EHDV 2	2	2 (2)	6.5+	20–80
		BTV 11	2	2 (1)	6.5+	10–20
		BTV 13	1	1	2.5	10
1986	2	EHDV 2	2	2 (2)	3.5–5.5	40
1987	3	EHDV 2	2	2 (2)	4.5–6.5+	40
		BTV 11	1	1	6.5+	10
1988	3	EHDV 2	1	1 (1)	3.5	320
		BTV 11	2	1 (1)	5.5–6.5+	80
		BTV 13	1	0	5.5	40
1989	4	EHDV 2	2	2 (2)	3.5–6.5+	40
		BTV 11	1	1	6.5+	10
		BTV 13	2	1 (1)	2.5–3.5	20–40
		BTV 17	1	0	2.5	10

[a] Number of tested deer with serum neutralizing antibodies to a single EHDV or BTV (Number with antibody titer ≥20).
[b] Age of deer with serum neutralizing antibodies.
[c] Underlined serotype meets criteria for previous exposure (monospecific reaction at ≥1:20).

west of Ossabaw Island, did not include *C. variipennis*; however, *C. insignus*, a species from which BTV has been recovered in Florida (Greiner et al., 1985), was collected.

The low prevalence of precipitating antibodies in cattle during the summer of 1981 is surprising since clinical disease was apparent in deer 1 yr before. Since there had been no significant additions to this cattle herd during this period, the low antibody prevalence is indicative of a low local rate of exposure during the previous years. Although this discrepancy may be directly related to exposure differences between host species, it is also possible that peak BTV activity on Ossabaw occurred during 1981, 1 yr after the onset of white-tailed deer mortality. From precipitating antibody prevalence data, peak EHDV activity appears to have occurred during 1982.

Our results suggest that precipitating antibodies as determined by the AGID test give a reasonable indicator of past exposure to BTV and EHDV in white-tailed deer populations but may result in an underestimation of prevalence. However, it is interesting how rapidly herd immunity disappears even in a population with an old-age structure as exists on Ossabaw Island. In areas of higher exploitation or rapid population turnover, herd immunity to these viruses without repeated exposure would be very short-lived.

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