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Source: Journal of Wildlife Diseases, 31(3) : 331-338

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-31.3.331

Journal of Wildlife Diseases, 31(3), 1995, pp. 331–338

EPIZOOTIC HEMORRHAGIC DISEASE VIRUS AND BLUETONGUE VIRUS SEROTYPE DISTRIBUTION IN WHITE-TAILED DEER IN GEORGIA

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ABSTRACT: Serum samples collected from 1,396 white-tailed deer (*Odocoileus virginianus*) in five areas of Georgia (USA) from 1989 to 1991 were tested for precipitating and serum neutralizing (SN) antibodies to the enzootic North American epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) serotypes. Precipitating antibodies to the EHDV or BTV serogroups, as detected by agar gel immunodiffusion (AGID) tests, were present in 35%, 29%, and 39% of deer sampled in 1989, 1990, and 1991, respectively. Significant differences ($P < 0.05$) in precipitating antibody prevalence were detected between physiographic regions during all years. Antibody prevalence consistently was highest in deer sampled from the Coastal Plain (77%), followed by the Piedmont (33%), Ridge and Valley (29%), Barrier Island (5%), and Blue Ridge (2%) regions. All AGID-positive samples were tested by SN tests for antibodies against all North American EHDV and BTV serotypes (EHDV serotypes 1 and 2, BTV serotypes 2, 10, 11, 13, and 17). Criteria for previous exposure to a specific serotype were either detection of monospecific results or clusters of positive results against that serotype. Serologic evidence of previous exposure to EHDV serotypes 1 and 2, and BTV serotypes 11 and 13 was detected during all years. Predominant serotypes varied among years. In general, evidence of exposure to EHDV serotype 2 appeared annually while exposure to BTV serotype 13 and EHDV serotype 1 decreased and increased, respectively. To determine serotype diversity prior to 1989, 134 AGID-positive white-tailed deer serum samples collected from 1967 to 1988 also were tested by SN. Evidence of exposure to EHDV serotypes 1 and 2 and BTV serotypes 11, 13, and 17 was detected.

Key words: Bluetongue viruses, epizootic hemorrhagic disease viruses, white-tailed deer, *Odocoileus virginianus*, serotype, Georgia, epizootiology.

INTRODUCTION

Hemorrhagic disease (HD), caused by viruses in either the epizootic hemorrhagic disease virus (EHDV) or bluetongue virus (BTV) serogroups (Reoviridae: Orbivirus), is the most important viral disease affecting white-tailed deer (*Odocoileus virginianus*) in the United States (Nettles and Stallknecht, 1992). In the United States, two EHDV serotypes (EHDV serotypes 1 and 2) and five BTV serotypes (BTV serotypes 2, 10, 11, 13, and 17) have been reported (Pearson et al., 1992). With the exception of BTV serotype 2, all of these serotypes have been associated with clinical HD in either experimental or natural infections of white-tailed deer (Shope et al., 1960; Thomas et al., 1974; Barber and Jochim, 1975; Howerth et al., 1988; Pearson et al., 1992; V. F. Nettles, unpubl.).

Hemorrhagic disease is a recurring problem in white-tailed deer in Georgia and other states in the southeastern United States (Nettles and Stallknecht, 1992). Although antibodies to EHDV and BTV serogroups have been reported from white-tailed deer from this region (Stallknecht et al., 1991a), very little work has been done to determine the specific virus serotypes responsible for this antibody response. Since more than one serotype can be present in a given area (Thomas et al., 1974) and changes in serotypes with time have been reported (Stott et al., 1981), information on serotype diversity and temporal patterns is critical to understanding herd immunity and disease.

Infections with specific EHDV and BTV serotypes can be confirmed only through virus isolation. In Georgia, however, there have been relatively few EHDV and BTV isolations from deer during the past 15 yr (Pearson et al., 1992), and in most cases, the causative virus(es) responsible for the

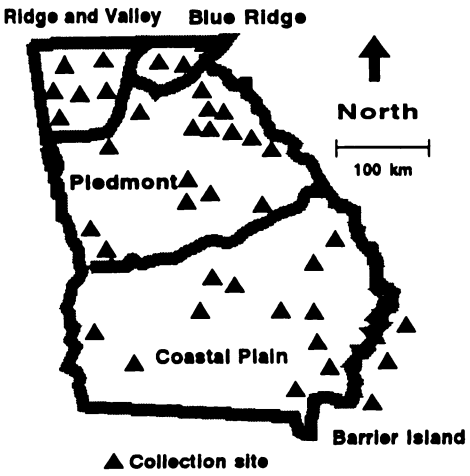

FIGURE 1. Physiographic regions of Georgia and white-tailed deer sample sites for 1989 to 1991.

repeated epizootics of HD during this period are unknown. Although serology can be used to determine exposure to specific EHDV and BTV serotypes, interpretation of results is complicated by cross neutralization which can occur between viruses within serogroups (Jochim, 1985). However, Taylor et al. (1985) proposed that with certain constraints in data interpretation, serum neutralization (SN) test results can be used to determine specific serotype exposure. This approach, which relies on detection of monospecific test results or clusters of positive results to individual EHDV or BTV serotypes, recently was used in a retrospective study of a 1981 epizootic of HD among white-tailed deer on Ossabaw Island, Georgia (Stallknecht et al., 1991b). In our current study, we used SN test results from white-tailed deer to determine the diversity and temporal distribution of EHDV and BTV serotypes in Georgia from 1989 to 1991. In addition, a retrospective study using banked white-tailed deer serum samples was done to determine the extent of change in these serotypes over time.

METHODS

Serum samples were collected from hunter-killed white-tailed deer from October to January during 1989, 1990, and 1991. Samples were collected from 40 areas representing five physiographic regions (Fig. 1). Most samples were collected on wildlife management areas administered by the Georgia Department of Natural Resources. Additional samples were collected by Southeastern Cooperative Wildlife Disease Study (SCWDS) personnel from hunter-killed deer from private properties or National Park Service lands. Age of deer was determined by tooth eruption and wear patterns (Severinghaus, 1949).

Serum samples tested in the retrospective study were collected from 1967 to 1988 and included hunter-killed deer and deer collected during August and September during herd health checks. All samples were stored in a serum bank at −20 C prior to testing.

Serum samples were screened for precipitating antibodies to the EHDV or BTV serogroups using agar gel immunodiffusion (AGID) tests as described by Pearson and Jochim (1979). Samples used in the retrospective study (pre-1989) had been tested previously by EHDV and BTV AGID tests at the Georgia Diagnostic Assistance Laboratory (College of Veterinary Medicine, The University of Georgia, Athens, Georgia) or at the National Veterinary Services Laboratories (NVSL) (Science and Technology, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa, USA). Serum samples collected during 1989, 1990, and 1991 were tested with commercial BTV and EHDV AGID test kits (Veterinary Diagnostic Technology, Inc., Wheat Ridge, Colorado, USA) as described by the manufacturer.

Serum neutralization tests were done on all AGID-positive samples. All serum dilutions, viral dilutions, and cell suspensions were made in a maintenance medium consisting of minimum essential medium with Earle's salts and sodium bicarbonate supplemented with 3% heat-inactivated fetal bovine serum and antibiotics (100 units penicillin, 0.1 mg streptomycin, and 0.25 μg amphotericin B/ml) (Sigma Chemical Company, St. Louis, Missouri, USA). All serum samples were diluted 1:5 in maintenance medium and heat-inactivated for 30 min at 55 C.

For initial screening, 25 μl of BTV serotypes 2, 10, 11, 13, and 17 and EHDV serotypes 1 and 2 (NVSL) at 100 to 300 median tissue-culture-infective doses ($TCID_{50}$)/25 μl were added to paired wells in a 96-well tissue culture plate. Each well then was inoculated with 25 μl of the serum dilution (final serum dilution 1:10). Two additional wells for each sample were run as a serum control and received 25 μl of maintenance medium and 25 μl of the serum dilution. Following incubation for 1 hr at 37 C, 150 μl of maintenance medium containing approximately 1.2×10^4 baby hamster kidney cells (BHK_{21}) (American Type Culture Collection,

TABLE 1. Prevalence of precipitating antibodies to eipzootic hemorrhagic disease or bluetongue viruses in white-tailed deer in Georgia, 1989 to 1991.

Year	Physiographic region					Total
	Blue Ridge	Ridge and Valley	Piedmont	Coastal Plain	Barrier Island	
1989	1/33 (3%)*	28/51 (55%)	47/171 (27%)	83/99 (84%)	7/119 (6%)	166/473 (35%)
1990	0/6 (0%)	14/82 (17%)	45/125 (36%)	55/81 (68%)	2/106 (2%)	116/400 (29%)
1991	0/2 (0%)	7/34 (20%)	64/174 (37%)	113/144 (78%)	11/142 (8%)	195/496 (39%)
Total	1/41 (2%)	49/167 (29%)	156/470 (33%)	251/324 (77%)	20/367 (5%)	477/1,369 (35%)

* Number positive in EHDV or BTV AGID tests/number tested (% positive).

Rockville, Maryland, USA) 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 >10% cytopathic effect were considered negative. All serum samples 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.

On each day of testing, 1:5 dilutions of seropositive bovine antisera against BTV serotypes 2, 10, 11, 13, and 17, and EHDV serotypes 1 and 2 (NVSL) were included as positive control samples. Negative controls also were included each day and consisted of a 1:5 dilution of heat-inactivated fetal bovine serum.

Evidence of prior exposure to a given serotype consisted of either a monospecific test result or the presence of clusters of seropositive results to a specific serotype (Taylor et al., 1985). For our study, the minimum positive titer for a monospecific reaction was raised from 1:10 to 1:20. A cluster was accepted as evidence of exposure if 50% or more of the positive serum samples from a given area (minimum of five positive samples) were positive to a specific serotype.

Prevalences of precipitating EHDV or BTV antibodies were tested for independence to physiographic region and year using chi-square tests (SAS Institute Inc., 1988).

RESULTS

From 1989 to 1991, serum samples were collected from 1,369 white-tailed deer. Prevalence of precipitating antibodies to viruses in the EHDV or BTV serogroups varied by year and physiographic region (Table 1). Differences ($P < 0.05$) between physiographic regions were detected in all years. During 1989, significant differences ($P < 0.05$) were detected in all pair-wise comparisons between physiographic regions, with the exception of the Barrier Islands and the Blue Ridge regions. During 1990 and 1991, antibody prevalence in the Coastal Plain deer was higher ($P < 0.05$) than that observed in the Blue Ridge, Ridge and Valley, Piedmont, and Barrier Islands regions. The Barrier Island antibody prevalence estimates also were lower ($P < 0.05$) than the antibody prevalences observed in deer from the Piedmont and Ridge and Valley regions. By year, significant differences were detected only between the 1990 (29%) and 1991 (39%) samples ($P < 0.001$).

Evidence of exposure to EHDV serotypes 1 and 2 and BTV serotypes 11 and 13 were detected during 1989, 1990, and 1991 (Table 2). Antibodies to EHDV serotype 2 were most common; more than 80% of seropositive deer tested positive to this serotype each year. Serologic evidence of EHDV serotype 1 exposure was limited to monospecific results from a single deer in both 1989 and 1990. In contrast, antibodies to EHDV serotype 1 were widespread during 1991 and were detected in 39% of all seropositive deer. Antibodies to BTV serotype 13 were present in 43% of seropositive deer during 1989, but prevalence of antibodies to this serotype decreased during 1990 and 1991. Evidence of previous exposure to BTV serotype 11 was limited to monospecific reactions in one, two, and one deer during 1989, 1990, and 1991, respectively. Based on the criteria of either monospecific results or clusters, no evidence of previous exposure to BTV serotypes 2, 10, or 17 was detected. Only 19 (5%) of 418 AGID-positive serum

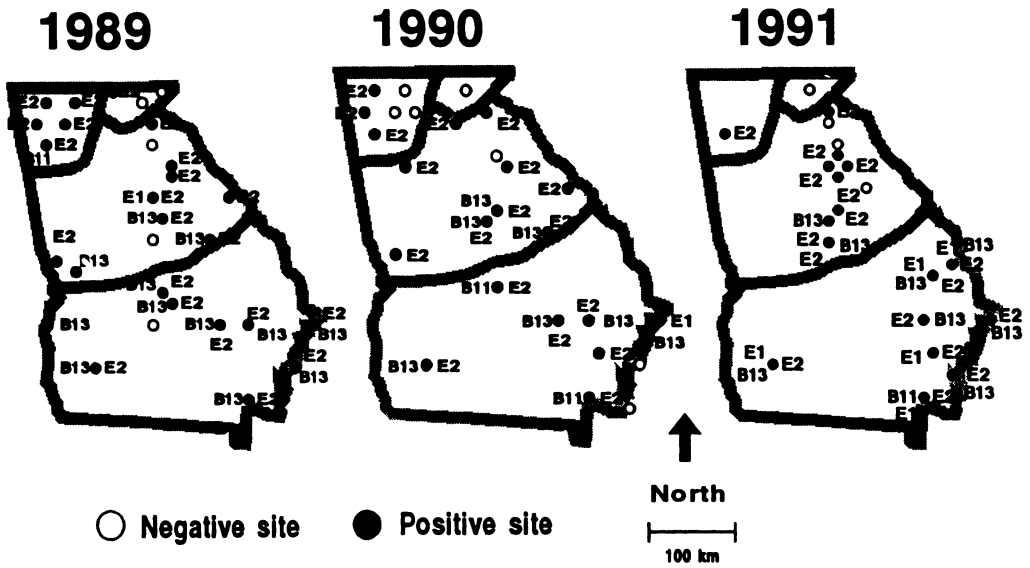

FIGURE 2. Distribution of epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) serotypes in Georgia as determined by serologic testing of white-tailed deer sampled from 1989 to 1991 (E1 = EHDV serotype 1, E2 = EHDV serotype 2, B11 = BTV serotype 11, B13 = BTV serotype 13).

samples tested by SN tests from 1989 to 1991, were negative for antibodies to any of the seven EHDV and BTV serotypes.

Serotype distribution varied by location (Fig. 2). Evidence of EHDV serotype 2 exposure was detected statewide, whereas exposure to EHDV serotype 1 and BTV serotype 13 was restricted to the Coastal Plain, Barrier Islands, and the lower portion of the Piedmont physiographic regions. Although evidence of previous exposure to BTV serotype 11 was minimal, monospecific results were detected at the same site during both 1990 and 1991.

Distributions of neutralizing antibodies by age-class and year were determined for EHDV serotype 2 and BTV serotype 13 from 1989 to 1991 (Fig. 3). Age class distributions for antibodies to EHDV serotype 1 were determined for 1991 only (Fig. 3), since antibodies to this serotype were detected in only six and nine deer during 1989 and 1990, respectively. These serotypes represented the predominant serotypes for which antibodies were detected from 1989 to 1990. For BTV serotype 13, positive results were detected in all adult age-classes (1.5, 2.5, and 3.5+ yr) during 1989. During 1990 and 1991, more than 90% of the deer with antibodies to BTV

TABLE 2. Neutralizing antibodies to North American epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) serotypes in white-tailed deer in Georgia from 1989 to 1991.

Year	Number[a]	Serotypes							
		EHDV-1	EHDV-2	BTV-2	BTV-10	BTV-11	BTV-13	BTV-17	Negative
1989	131	6 (5%)[b,c]	105 (80%)[c]	10 (8%)	11 (8%)	11 (8%)[c]	57 (43%)[c]	8 (6%)	3 (2%)
1990	108	8 (7%)[c]	98 (91%)[c]	3 (3%)	2 (2%)	6 (6%)[c]	27 (25%)[c]	5 (5%)	5 (5%)
1991	179	64 (39%)[c]	154 (86%)[c]	11 (6%)	8 (4%)	10 (6%)[c]	47 (26%)[c]	11 (6%)	11 (6%)

[a] Number of AGID-positive (EHDV or BTV) serum samples tested by serum neutralization.
[b] Number positive at 1:10 dilution (% positive).
[c] Positive results meet criteria for previous exposure to this serotype (monospecific reactions or clusters).

serotype 13 were associated with the 2.5 and 3.5+ yr age-classes. Antibodies to EHDV serotype 2, however, were distributed more evenly among age-classes during all years. Antibodies to EHDV serotype 1 also were distributed more evenly among adult age-classes during 1991 when the prevalence of antibodies to this serotype greatly increased.

Retrospective SN tests on AGID-positive samples from 1967 to 1988 provided evidence of previous exposure to EHDV serotypes 1 and 2, and BTV serotypes 11, 13, and 17 (Table 3). Evidence of EHDV serotype 2 exposure was detected during all years; all other serotypes appeared intermittently. No evidence of exposure to BTV serotypes 2 or 10 was detected.

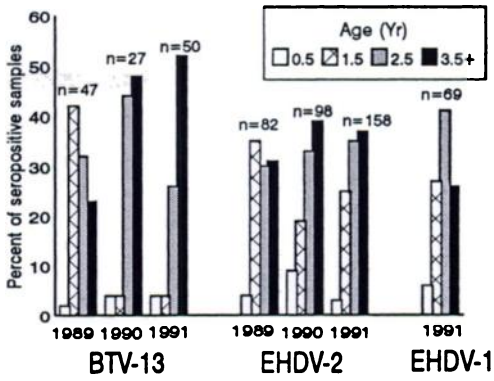

FIGURE 3. Distribution of serum neutralizing antibodies to bluetongue virus (BTV) serotype 13 and epizootic hemorrhagic disease virus (EHDV) serotypes 1 and 2 in white-tailed deer in Georgia by age-class from 1989 to 1991.

DISCUSSION

Results from AGID tests from deer sampled in Georgia during 1989 to 1991 were consistent with AGID results from 1981 to 1988 (Stallknecht et al., 1991a). Prevalence of precipitating antibodies to viruses in the EHDV or BTV serogroups was highest in deer from the Coastal Plain, followed by the Piedmont, Ridge and Valley, Barrier Islands, and Blue Ridge regions. Annual trends from 1989 to 1991, however, varied by region. In the southern part of Georgia (Piedmont, Coastal Plain, and Barrier Islands), antibody prevalence increased from 33% in 1990 to 41% in 1991 ($P < 0.05$). In the mountain regions (Ridge and Valley and Blue Ridge), although not statistically significant, a continuous decline in antibody prevalence was observed from 1989 to 1991. This decline can be attributed to the disappearance of residual antibodies from a 1988 HD epizootic in these regions (Nettles and Stallknecht, 1992). During this epizootic, clinical disease was observed in deer from both of these physiographic regions and EHDV serotype 2 was confirmed by virus isolation (Nettles and Stallknecht, 1992).

Serotype diversity present in Georgia varied by year, as has been reported in the western United States (Stott et al., 1981), Central America and the Caribbean (Homan et al., 1990), and Australia (Gard and

TABLE 3. Neutralizing antibodies to North American epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) serotypes in white-tailed deer in Georgia, 1967 to 1988.

Years	Number[a]	Serotypes						
		EHDV-1	EHDV-2	BTV-2	BTV-10	BTV-11	BTV-13	BTV-17
1967–1970	23	13 (57%)[b,c]	19 (83%)[c]	0	0	0	0	1 (4%)
1978–1980	29	0	28 (97%)[c]	1 (3%)	1 (3%)	2 (7%)	5 (17%)[c]	8 (28%)[c]
1981–1982	22	13 (59%)[c]	12 (55%)[c]	2 (9%)	0	0	4 (18%)[c]	5 (23%)[c]
1983–1984	11	8 (73%)[c]	6 (55%)[c]	0	0	0	3 (27%)[c]	0
1985–1986	18	6 (33%)	16 (89%)[c]	1 (6%)	0	0	2 (11%)	1 (6%)
1987–1988	31	5 (16%)	30 (97%)[c]	0	0	4 (13%)[c]	6 (19%)[c]	1 (3%)
Total	134	45 (34%)	111 (83%)	4 (3%)	1 (1%)	6 (4%)	20 (15%)	16 (12%)

[a] Number of AGID-positive (EHDV or BTV) samples tested by serum neutralization.
[b] Number positive at 1:10 dilution (% positive).
[c] Results meet criteria for previous exposure to this serotype (monospecific results or clusters).

Melville, 1992). This variation was apparent in both the current and retrospective data. Evidence of EHDV serotype 2 exposure was detected every year, whereas other serotypes appeared intermittently (EHDV serotype 1, BTV serotypes 11, 13, and 17) or not at all (BTV serotypes 2 and 10). It is interesting that a similar relationship was observed in Australia where virus isolation attempts from sentinel cattle resulted in the isolation of eight BTV serotypes and four EHDV serotypes (Gard and Melville, 1992). All of these serotypes appeared intermittently from 1981 to 1990, except EHDV serotype 2, which was isolated each year from 1984 to 1990.

Regional variation in serotype distribution also was apparent in Georgia from 1989 to 1991. Serotype diversity was greatest in the lower Piedmont, Coastal Plain, and Barrier Islands, with evidence of previous exposure to multiple serotypes detected each year. Diversity decreased in the northern Piedmont, Ridge and Valley, and Blue Ridge regions where, with few exceptions involving monospecific reactions from individual animals, EHDV serotype 2 was the only serotype present.

Based on age distribution of antibodies (Fig. 3) and changes in overall antibody prevalence to specific serotypes (Table 2), we believe that two major serotype changes occurred from 1989 to 1991. Exposure to BTV serotype 13 had a decreasing trend with little or no evidence of exposure during 1990 and 1991. In contrast, EHDV serotype 1 exposure increased dramatically in 1991; this is evidence for the widespread emergence of this serotype during that year.

There was good agreement between serotypes identified serologically in this study and reported virus isolation from white-tailed deer in Georgia and adjacent states. This concordance was apparent in both the retrospective and current data. During 1978 to 1980, BTV serotype 17 and several untyped EHDV's were isolated from deer in Alabama and Georgia (Pearson et al., 1992). From 1981 to 1982, BTV serotypes 11 and 17 and EHDV serotypes 1 and 2 were isolated from deer in Georgia (Odiawa et al., 1985). Although BTV serotype 11 was not detected in our serologic data during those years (Table 3), monospecific antibodies to BTV serotype 11 were detected on Ossabaw Island where it represented the dominant serotype in deer sampled during 1981 and 1982 (Stallknecht et al., 1991b). No viral isolations are reported from 1983 to 1987. In 1988, however, EHDV serotype 2 was isolated in Georgia, Alabama, and Tennessee (USA), BTV serotype 13 was isolated in Georgia, and BTV serotype 11 was isolated from South Carolina (USA) (Pearson et al., 1992). These are the same serotypes detected in our current study which began 1 yr later. From 1989 to 1991, EHDV serotype 2 was isolated from deer in Alabama during 1989 and from deer in Georgia during 1990 (Pearson et al., 1992). No isolations of BTV were reported from 1989 to 1991. In 1991, EHDV serotype 1, which was serologically detected in Georgia, was isolated from deer in Tennessee (D. E. Stallknecht, unpubl.).

The factors responsible for HD epizootics in white-tailed deer populations have not been defined. Based on our results it is evident that herd immunity to individual serotypes can vary greatly over time. In white-tailed deer, it appears that immunity to these viruses does not cross-protect between viruses in different serogroups (Hoff and Trainer, 1974). There is some evidence from other species, however, that protection can extend between serotypes within the same serogroup, especially under conditions of multiple exposures (Jeggo et al., 1984). Results from testing of serum samples collected during major HD epizootics in white-tailed deer in Georgia during 1980 and 1988 (Nettles and Stallknecht, 1992) are consistent with this. During both of these years, serology and reported virus isolations provide evidence for the presence of both EHDV and BTV serotypes. The intermittent nature of activity of the BTV serotypes, which also is supported by both serology and reported

virus isolation results, is evidence that these viruses emerged when there was little or no herd immunity to any of BTV serotypes. In 1988, both EHDV serotype 2 and BTV serotype 13 were isolated from deer (Pearson et al., 1992). In the case of EHDV serotype 2, the epizootic extended into the upper Piedmont, Ridge and Valley, and Blue Ridge regions where few deer had previous exposure to any of the EHDV or BTV serotypes (Stallknecht et al., 1991a). In contrast, few reports of morbidity or mortality were associated with the widespread emergence of EHDV serotype 1 during 1991 in the lower Piedmont and Coastal Plain of Georgia, where previous exposure and resultant herd immunity to EHDV serotype 2 was high.

Although problems with specificity of serologic testing for antibodies to EHDV and BTV occur at both the serogroup and serotype level, the AGID tests supported by SN tests and interpreted within the constraints used in this study, can provide a very effective tool for large-scale epizootiologic studies of these viruses. Serology has advantages over virus isolation in its simplicity, field adaptability, cost efficiency, and suitability for large-scale surveillance. However, because antibodies persist and may not represent recent exposure, this type of surveillance program does require age data on tested animals, several years of data to document changes in dominant serotypes, or both in order to understand when and if exposure occurred during a given time period. Since antibody prevalence to individual serotypes may be low, serologic surveillance also requires a relatively large sample size. Finally, because virus isolation still provides the only confirmation of the presence of a specific virus, isolation attempts should be integrated into any surveillance program whenever possible to validate serologic results.

ACKNOWLEDGMENTS

This project was supported through an appropriation from the Congress of the United States to the Southeastern Cooperative Wildlife Disease Study, Department of Parasitology, College of Veterinary Medicine, The University of Georgia, which was administered and coordinated under Federal Aid in Wildlife Restoration Act (50 Stat 917). Additional support was provided through Grant Agreement Number (14-16-000491-913), Fish and Wildlife Service, U.S. Department of the Interior, and through Cooperative Agreement Number (12-34-93-032), Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture. Sincere appreciation is expressed to personnel of the Game and Fish Division, Georgia Department of Natural Resources, for their long-term support and assistance with this project.

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Received for publication 6 April 1994.