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Antibodies to Bluetongue and Epizootic Hemorrhagic Disease Viruses from White-Tailed Deer Blood Samples Dried on Paper Strips

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ABSTRACT: The feasibility of using dried blood samples for serologic testing of white-tailed deer (*Odocoileus virginianus*) for antibodies to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) was tested with matched samples of serum and eluted dried whole bood. Results from matched serum virus neutralization (SN) tests indicated that a 1-ml elution from a 1- × 2-cm section of filter paper strip containing dried blood approximated a 1:10 serum dilution. Neutralizing antibody titers detected from 34 matched titrations of serum and dried blood samples were equivalent in 25 (74%) titrations and were within a single dilution in the remaining nine (26%) titrations. Eluted blood samples from SN-positive deer, however, did not produce detectable precipitin lines on agar gel immunodiffusion tests for antibodies to either BTV or EHDV. In a trial using serum and dried blood samples from 108 hunter-killed deer from five locations in Georgia (USA), antibody prevalence and serotype distribution results were similar. Use of dried blood samples for serologic testing for antibodies to BTV and EHDV provides a reliable alternative to serum but should be considered only when serum collection is not feasible.

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

Use of dried blood for serologic testing is well documented and the technique has been adapted to numerous serological tests, including hemagglutination inhibition (HI), indirect fluorescent antibody, serum virus neutralization (SN), enzyme-linked immunosorbent assay (ELISA), and complement fixation (Adams and Hanson, 1956; Karstad et al., 1957; Benson and Mickle, 1963; Brody et al., 1964; Gaggero and Sutmoller, 1965; Nobuto, 1967; Kimber and Burridge, 1972; Wolff and Hudson, 1974; Beard and Brugh, 1977; Brugh and Beard, 1980; Young and Purnell, 1980; Hamblin and Hedger, 1982). Advantages of this technique for blood collection include simplicity of collection and handling of samples, the need for minimal personnel training, decreased blood volume requirements, and increased ability to collect samples from dead animals (Wolff and Hudson, 1974). These advantages make this technique particularly appealing for use in serological surveillance of wildlife populations. The objective of this study was to compare the antibody titers to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) in matched serum and eluted dried whole blood samples from white-tailed deer (*Odocoileus virginianus*), for the purpose of testing the feasibility of using dried whole blood samples to determine the presence of BTV and EHD antibodies.

Matched serum and dried whole blood samples were collected from deer sampled during herd health checks in Georgia and Louisiana (USA) during August and September, 1990, and from hunter-killed deer harvested on five wildlife management areas in Georgia during the 1990 hunting season. Blood was collected either from the heart immediately after death (herd health checks) or from the thoracic inlet or cavity (hunter-killed deer) within 5 hr of death. Serum samples were stored at −20 C until testing. For dried blood samples, unclotted whole blood was absorbed on a 2.0 × 6.0 cm paper strip (paper #740E, Schleicher and Schuell Inc., Leene, New Hampshire, USA). Strips were immersed in blood for approximately 10 sec, and were air dried at room temperature for 12 to 24 hr. Strips were stored in individual sealed 50-ml plastic tubes at 4 C until testing. All testing

was completed within 2 mo of blood collection.

Optimum strip size was determined using seven matched serum and dried blood samples collected from deer on herd health checks. All serum samples tested positive for antibodies to either BTV or EHDV in agar gel immunodiffusion (AGID) tests (Pearson and Jochim, 1979). Three sizes of paper strips were tested: 0.5 × 2.0 cm, 1.0 × 2.0 cm, and 1.5 × 2.0 cm. These sizes were selected based on the protocol reported by Brugh and Beard (1980) in which two 4.8 mm diameter disks in 0.2 ml phosphate buffered saline solution (PBS) (1.8 cm^2/ml PBS) approximated a 1:10 serum dilution in an HI test for Newcastle disease virus antibodies. For elution of antibodies, strips with absorbed dried blood were cut into four equal pieces and were placed in a single tube containing 1 ml of sterile Dulbecco's PBS (D-PBS) (pH = 7.2). Strips in D-PBS were stored for 24 hr at 4 C. Prior to testing, the eluted blood was centrifuged for 10 min at 1,500 × g to remove particulates.

Matched serum and eluted blood samples were tested by SN against BTV 2, 10, 11, 13, and 17 and EHDV 1 and 2 as described (Stallknecht et al., 1991). Serum samples were initially tested at a 1:10 dilution. Serum samples neutralizing virus at this dilution were further tested at dilutions ranging from 1:10 to 1:320. Test protocols were identical for eluted blood samples. However, eluted blood samples were initially screened against the BTV and EHDV serotypes at a 1:2 dilution. All samples that neutralized virus were tested against the respective serotypes at dilutions ranging from 1:2 to 1:64. Eluted blood samples also were tested for precipitating antibodies to both BTV and EHDV using the AGID tests.

Based on this initial trial, we concluded that a 1.0 × 2.0 cm portion of the strip in 1 ml D-PBS approximated a 1:10 serum dilution (Table 1). Results from serum and eluted blood samples were similar in nine of ten titrations compared to six of ten for the 0.5 × 2.0 cm strip and two of ten for the 1.5 × 2.0 cm strip. Therefore, all further testing was done with the 1.0 × 2.0 cm strip. Precipitating antibodies were not detected in any of the eluted blood samples tested by AGID.

In order to verify this agreement, 24 additional matched titrations to BTV 13, EHDV 1 and EHDV 2 were completed on the remaining AGID-positive serum samples collected during herd health checks. When combined with results from the previous 10 matched titrations (Table 1), serum and eluted dried blood (×10) antibody titers were identical in 25 of 34 (74%) titrations and were within a single twofold dilution for the remaining nine (26%) titrations (Table 2). This agreement was independent of antibody titers which ranged from 1:10 to 1:320 for these serum samples.

Since all of these initial matched SN tests were done in parallel on the same 96-well plate, a blind trial utilizing matched serum and dried-blood samples collected from hunter-killed deer was conducted. Matched samples were collected from 108 deer at five locations in Georgia and were tested independently. In this trial, all serum samples were screened for antibodies to BTV and EHDV using AGID tests. Serum samples testing positive with the AGID tests were further tested by SN. Since eluted blood from seven known AGID positive deer failed to produce detectable precipitin lines on AGID, eluted dried-blood samples from all deer were initially screened by SN at a 1:2 dilution. As before, samples which neutralized virus were tested against the respective serotype at dilutions ranging from 1:2 to 1:64.

Antibodies to BTV and EHDV were detected in deer from three of five locations by both techniques (Table 3). Serotypes present at these locations also were similar, as determined by monospecific SN tests for single BTV or EHDV serotypes. The only exception was the detection of antibodies to BTV 2 from a dried blood sample in location 2, but not from the matched se-

TABLE 1. Determination of optimum paper strip size for detection of virus neutralizing antibodies to bluetongue and epizootic hemorrhagic disease viruses from dried blood samples from white-tailed deer.

Sample number	Serotype	Serum antibody titer	Dried blood antibody titer (×10)		
			0.5 cm[a]	1.0 cm	1.5 cm
1	EHDV-2	1:40	1:40	1:40	1:80
2	EHDV-2	1:40	1:40	1:40	1:80
3	EHDV-1	1:320	1:320	1:320	1:640
	EHDV-2	1:20	<1:20	1:20	cr[b]
4	EHDV-1	1:10	<1:20	<1:20	1:20
	EHDV-2	1:40	<1:20	1:40	1:40
5	EHDV-2	1:20	<1:20	1:20	1:40
	BTV-11	1:40	1:40	1:40	1:80
6	EHDV-2	1:10	<1:20	<1:20	<1:20
7	EHDV-2	1:320	1:160	1:160	1:160

[a] Length of strip, width = 2.0 cm.
[b] Could not read plate.

rum sample (Table 3). Antibodies to BTV 2 in this dried blood sample were detected only at the initial 1:2 dilution.

Antibody prevalence estimates by location varied slightly between techniques, but in all there was 94% agreement between positive and negative SN results observed from serum versus eluted dried blood for both BTV and EHDV. The Kappa values, which account for chance in test comparisons, were 0.72 for EHDV and 0.68 for BTV, indicating a high level of agreement between test results (Martin et al., 1987).

Most variation in the antibody prevalence estimates among serum and dried whole-blood samples (Table 3) was attributable either to not detecting precipitating antibodies in the initial AGID tests or to low BTV antibody titers. Our AGID screening tests failed to detect antibodies in four serum samples from location 2 which were SN positive to EHDV 2 upon subsequent testing. The low BTV antibody prevalence detected from dried blood samples at location 3 can be explained by the higher minimum detectable dilution (equivalent to a 1:20 serum dilution) required with this technique. Of the 10 SN-positive serum samples detected at location 3, four were positive at the 1:10 dilution and two were positive at the 1:20 dilution.

Use of dried blood samples for serologic testing for antibodies to BTV and EHDV provides an acceptable alternative to serum testing. However, due to decreased sensitivity and the increased laboratory time required, use of dried-blood samples is recommended only when collection and testing of serum is not feasible.

Although beyond the scope of this study, antibody levels in dried blood specimens are relatively stable for at least 60 days,

TABLE 2. Virus neutralizing antibody titers to bluetongue and epizootic hemorrhagic disease viruses on matched serum and eluted dried blood samples from white-tailed deer.

Serum antibody titer	Number of samples positive	Antibody titer observed in matched eluted blood samples[a]		
		−1	0	+1
1:10	5		4	1
1:20	6	1	4	1
1:40	13	2	10	1
1:80	4		4	
1:160	3		1	2
1:320	3	1	2	

[a] Number of dried blood samples testing seropositive at one two-fold dilution above (+), equal to (0), or below (−) corresponding serum neutralizing antibody titer.

TABLE 3. Virus neutralizing antibodies to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) in hunter-killed white-tailed deer from five locations in Georgia, as determined from serum and eluted dried blood samples.

Location	Number of samples	BTV results[a]		EHDV results		Serotypes[b]	
		Serum	Dried blood	Serum	Dried blood	Serum	Dried blood
1	9	0	0	0	0	—	—
2	22	2 (9%)	2 (9%)	12 (55%)	16 (73%)	BTV-13 EHDV-2	BTV-2 BTV-13 EHDV-2
3	19	10 (53%)	7 (37%)	12 (63%)	11 (58%)	BTV-13 EHDV-1 EHDV-2	BTV-13 EHDV-1 EHDV-2
4	44	1 (2%)	1 (2%)	0	0	BTV-13	BTV-13
5	14	0	0	0	0	—	—

[a] Number seropositive (% seropositive).
[b] Monospecific antibodies detected to these serotypes.

especially under refrigerated conditions (Gaggero and Sutmoller, 1965; Brugh and Beard, 1980). Effects of long-term storage (>60 days) still need to be evaluated. In addition to the SN tests described here, use of eluted dried blood also lends itself to ELISA technology (Young and Purnell, 1980; Hamblin and Hedger, 1982), which may provide a more reliable serologic test for group specific antibodies to these viruses in the near future (Reddington et al., 1991).

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