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Serologic Detection of Bluetongue Virus Infection of Black-tailed Deer: Comparison of Serum Neutralization, Agar Gel Immunodiffusion, and Competitive ELISA Assays

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ABSTRACT: Three adult black-tailed deer (*Odocoileus hemionus columbianus*) and four fawns were inoculated with bluetongue virus (BTV) serotype 10 or 17, or epizootic hemorrhagic disease virus (EHDV) serotype 1. Animals were bled at irregular intervals thereafter and the presence of virus-specific antibodies in serum determined by agar gel immunodiffusion (AGID), serum neutralization (SN) and competitive enzyme-linked immunosorbent assay (C-ELISA) tests. Serum antibodies to BTV were detected in all three tests for 692 days after inoculation (DAI) of adult deer, but both the SN and AGID tests gave either erroneous or misleading results. Serum from one deer was negative by the AGID test at 409 DAI with BTV-10 but was positive at 248 and 692 DAI; also one adult and one fawn had antibodies by the SN test to serotypes of BTV with which they were not inoculated. The AGID test for EHDV had false positive results with some sera from animals inoculated only with BTV, and it consistently had false negative results with serum samples collected from an EHDV-inoculated deer at 140 DAI and thereafter. The C-ELISA was the most useful test for the detection of antibodies to BTV because it rapidly gave quantitative and accurate results.

Key words: Black-tailed deer, *Odocoileus hemionus columbianus*, serology, bluetongue virus, epizootic hemorrhagic disease virus.

Bluetongue is an insect-transmitted viral disease of domestic and wild ruminants. Bluetongue virus (BTV), and the closely related epizootic hemorrhagic disease virus (EHDV), may cause severe disease in white-tailed deer (*Odocoileus virginianus*), pronghorn (*Antilocapra americana*), and bighorn sheep (*Ovis canadensis* (Robinson et al., 1967; Howerth et al., 1988; Thorne et al., 1988). Bluetongue virus infection of wild ungulates can result in a wide variety of signs, ranging from sudden death to chronic disease, but often infection is asymptomatic. Thus, activity of BTV and EHDV among populations of wild ruminants is most readily monitored by serological surveillance. The most widely used test, the agar gel immunodiffusion (AGID) test, is used to detect antibodies to group-specific antigens of BTV and EHDV which are common to all serotypes of each virus. Della-Porta et al. (1985) proposed that the AGID test is neither sufficiently sensitive nor specific to justify its continued use as the standard regulatory test for serological detection of BTV infection of domestic ruminants. The serum neutralization (SN) test usually is considered the most sensitive and specific of the available tests but is not practical for routine serological studies because it is time consuming, expensive and labor intensive. Furthermore, 24 serotypes of BTV and 10 serotypes of EHDV occur worldwide (Gorman, 1992), including four serotypes of BTV (BTV-10, BTV-11, BTV-13, and BTV-17) and two of EHDV (EHDV-1 and EHDV-2) in the western United States (Pearson et al., 1992). Thus, each serum must be titrated against several viruses. Several blocking or competitive enzyme-linked immunosorbent assays (C-ELISA) for detection of serum antibodies to BTV group-specific antigens recently have been described (Afshar et al., 1989; Reddington et al., 1991).

Bluetongue virus infection of wild ruminants is common in California (USA) and there is circumstantial evidence that it may be a significant cause of disease in black-tailed deer (Jessup et al., 1984, 1990).

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TABLE 1. Serologic detection of bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) infection of California black-tailed deer, 1989 to 1990.

Deer #	Inoculum	Serologic test								
		BTV AGID[a]	EHDV AGID	BTV C-ELISA[b]	Serum neutralization					
					BTV-10[c]	BTV-11	BTV-13	BTV-17	EHDV-1	EHDV-2
1	BTV-17	+[d]	−	+	−	−	−	+	−	−
2	BTV-17	+	+	+	−	+	+	+	−	−
136	EHDV-1	−	+	−	−	−	−	−	+	−

[a] AGID = agar gel immunodiffusion test.
[b] C-ELISA = competitive enzyme-linked immunosorbent assay.
[c] Serotype of BTV used in serum neutralization test.
[d] +, specific antibodies detected; −, no specific antibodies detected.

Our objective was to compare the SN, AGID, and C-ELISA tests for serological detection of BTV infection of black-tailed deer (*Odocoileus hemioneus columbianus*).

Sera were obtained from three adult black-tailed deer and four fawns; the study was conducted from January 1989 through 1990. All animals were hand-raised and housed throughout the study in insect-secure facilities according to the University of California animal care and use protocol. Detailed descriptions of the deer, including their maintenance in insect-secure isolation facilities and the experimental procedures, are described by Work et al. (1992). The animals were confirmed initially to be free of BTV and EHDV infection by virus isolation using embryonated chicken eggs (Goldsmith and Barzilai, 1968), and were seronegative to these two viruses as determined by AGID and C-ELISA tests. All deer were inoculated with either BTV or EHDV. Briefly, deer #1, an adult male, was intravenously (IV) inoculated with cell culture-derived BTV-17; deer #2, an adult female, was inoculated both IV and subcutaneously (SC) with sheep blood which contained BTV-17; deer #136, an adult male, was inoculated SC and intradermally (ID) with sheep blood and cell culture fluid, both of which contained EHDV-1. Blood was collected from these deer at varying intervals for <692 days after inoculation (DAI). Two male fawns (deer #4 and #5) were inoculated SC and ID with deer blood which contained BTV-17. An additional male fawn (deer #9) was inoculated SC with BTV-17-infected blood from an experimentally infected white-tailed deer. A female fawn (deer #8) was inoculated SC with BTV-10-infected blood that also was collected from a BTV-inoculated white-tailed deer. All fawns were killed at 12 or 13 DAI.

A microtiter SN test was used to detect neutralizing antibodies to BTV serotypes 10, 11, 13, and 17, and EHDV serotypes 1 and 2, essentially as described by Heidner et al. (1988); we used a challenge inoculum of approximately 250 tissue culture infectious doses ($TCID_{50}$) of each virus, and an initial serum dilution of 1:10. Titers are reported as the inverse of the final serum dilution which protected greater than 50% of the cell monolayer when cytopathic effect was complete (100%) in control wells. The AGID assays for BTV (Veterinary Diagnostic Technology, Wheat Ridge, Colorado, USA) and EHDV (National Veterinary Services Laboratory, Ames, Iowa, USA) were done as described by Pearson and Jochim (1979). The C-ELISA for detection of serum antibodies to BTV (Blueplate Special, DiagXotics, Wilton, Connecticut, USA) was used according to the manufacturer's instructions.

All three deer inoculated with BTV or EHDV developed antibodies to these viruses as determined by one or more of the three tests used (Table 1). Antibodies to BTV were detected in the C-ELISA, AGID

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and SN tests in sera from deer #1 and 2 <692 DAI. The AGID test gave a false negative result with serum collected from deer #1 at 409 DAI; this sample was positive by both the SN and C-ELISA tests. Although the SN test is considered the most sensitive and specific test for the serological diagnosis of BTV infection, these results are evidence that the significance of low SN titers to BTV may be difficult to interpret. For instance, deer #2 received only BTV-17 yet it also developed low SN antibody titers to BTV-11 and BTV-13. These results are consistent with a previous report that cattle inoculated with one or more BTV serotypes may develop antibody titers to virus serotypes to which they were never exposed (Thomas, 1985).

The AGID test for EHDV lacked both specificity and sensitivity, making it unreliable as a diagnostic test. Antibodies were not detected by the AGID test at 140 DAI or thereafter in deer #136 which was inoculated with EHDV-1, despite the presence of SN titers to EHDV-1 through 423 DAI when the animal was euthanized by an intravenous injection of T-61 (American Hoechts Corporation, Sommerville, New Jersey, USA). Deer #2 seroconverted to EHDV by AGID, although the animal was not exposed to EHDV as confirmed by the lack of seroconversion by SN test.

All four fawns developed antibodies to BTV by 12 DAI by at least one test (Table 2). Two fawns (deer #4 and #9) developed SN antibodies only to the serotype of BTV with which they were inoculated, whereas serum at 13 DAI from one fawn (deer #8) neutralized both BTV-10 and BTV-13. Interestingly, this animal developed SN antibodies to BTV-13 at 9 DAI whereas antibodies to BTV-10 (with which it was inoculated) were not detected until 13 DAI. One fawn (deer #4) developed antibodies to EHDV by the AGID test at 12 DAI, although the animal was not exposed to this virus and did not seroconvert to EHDV by SN test. These data are consistent with those obtained from adults, and are evidence that BTV-infected black-tailed deer can develop both cross-reactive SN antibodies to BTV as well as false positive antibody titers as determined by the EHDV AGID test.

TABLE 2. Serologic response of black-tailed deer fawns to bluetongue virus (BTV) infection.

Deer #	Inoculum	Antibodies to BTV (DAI)[a]		
		AGID[b]	SN[c]	C-ELISA[d]
4	BTV-17	12	12	12
5	BTV-17	12	—[e]	—
8	BTV-10	9	13	9
9	BTV-17	12	12	12

[a] Days after inoculation (DAI) of BTV when fawns first had antibodies to BTV.
[b] AGID = agar gel immunodiffusion test.
[c] SN = serum neutralization test using the same serotype of BTV as that used to inoculate the fawn.
[d] C-ELISA = competitive enzyme-linked immunosorbent assay.
[e] —, fawn had no detectable antibodies to BTV by this test at the time it was euthanized; this was 12 DAI for all animals except deer #8 which was euthanized at 13 DAI.

Serological responses of fawns to BTV infection differed. One fawn (deer #5) seroconverted to BTV by AGID test at 12 DAI, at which time it was negative by both C-ELISA and SN assays. Another (deer #8) seroconverted by both C-ELISA and AGID at 9 DAI, but antibodies were not detected by SN test until 13 DAI. Thus individual animals developed antibodies at different intervals after inoculation of BTV, depending on the assay used, as reported by others (Afshar et al., 1989; Reddington et al., 1991).

Exposure of free-ranging populations of ruminants to orbiviruses such as BTV and EHDV is most readily monitored by serological testing. While only a limited number of sera were evaluated in this study, we conclude that the C-ELISA is the most useful of the three tests evaluated. The C-ELISA was very accurate and, unlike the AGID test, the C-ELISA provided quantitative results, so that the strength of the serological response was accurately measured. The disadvantages of the C-ELISA are that it requires some specialized equipment and that single serum samples must be run in batches for economy. A

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C-ELISA specific for EHDV which could be used in conjunction with the BTV C-ELISA would improve serological diagnosis of orbivirus infections of free-ranging ruminants. Although a C-ELISA for EHDV has been developed (White et al., 1991), it is not commercially available.

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