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Determining Prevalence of Bluetongue and Epizootic Hemorrhagic Disease Viruses in Mule Deer in Arizona (USA) Using Whole Blood Dried on Paper Strips Compared to Serum Analyses

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ABSTRACT: We investigated the feasibility of using whole blood dried on paper strips as a means to collect antibody prevalence data for the epizootic hemorrhagic disease viruses (EHDV) and bluetongue viruses (BTV) from hunter-harvested male mule deer (*Odocoileus hemionus*) in October 2002 from Arizona, USA. We compared antibody prevalence estimates in mule deer from paired paper strip and serum samples. Prevalence data obtained from elution of dried blood on paper strips proved to be consistent with results from serum in 94% of the samples tested. The paper strip method allows easy collection of blood from dead animals, with a smaller amount of blood being needed for analyses. Also, samples do not need to be refrigerated before analyses. We also used serum samples to determine hemorrhagic disease (HD) serotype exposure status of mule deer harvested from 4 distinct areas in Arizona. Antibodies to BTV and EHDV were identified in 3 of the 4 areas, with positive results to EHDV-1, EHDV-2, BTV-10, and BTV-11 being most common. Many animals did not have antibodies against the BTV serotypes. Exposure varied geographically and potentially with elevation. Hemorrhagic disease viruses commonly infect Arizona mule deer, except on the Kaibab Plateau in northern Arizona.

Key words: Arizona, bluetongue virus, dried blood, epizootic hemorrhagic disease virus, mule deer, *Odocoileus hemionus*, serology.

Hemorrhagic disease (HD), caused by related viruses in the epizootic hemorrhagic disease and bluetongue virus serogroups (*Orbivirus, Reoviridae*), has been documented in free-ranging ruminants in North America since 1955 (Nettles, 1992; Stallknecht and Davidson, 1992), but only recently has the disease been recognized in Arizona, USA (Noon et al., 2002a, b). In 2001, two mule deer (*Odocoileus hemionus*) found near Prescott in central Arizona died of hemorrhagic disease; however, the geographic range of HD viruses in Arizona is unknown.

Hemorrhagic disease viruses have resulted in large-scale mortality in white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), and pronghorn (*Antilocapra americana*) (Thorne, 1982; Gibbs and Greiner, 1989), and as a result, serologic surveillance for these pathogens in free-ranging wildlife is warranted. Serologic data are typically acquired from serum obtained from whole, fresh blood (Trainer and Jochim, 1969; Stallknecht et al., 1995). However, the efficacy of dried whole blood samples collected on paper strips for HD surveillance in free-ranging white-tailed deer has been demonstrated elsewhere (Stallknecht and Davidson, 1992). Paper strip samples are easier to collect, handle, and store than whole blood, which must be centrifuged and refrigerated until analysis. Other advantages of paper strips include the smaller volume of blood that is required and the ability to collect usable samples from dead animals. These characteristics make paper strips an attractive option for sampling free-ranging wildlife, particularly those taken by hunters. The objectives of our study were: 1) to test the paper strip method of blood collection in free-ranging, hunter-harvested male mule deer in Arizona, and 2) to determine the geographic distribution of HD viruses among distinct mule deer populations in Arizona.

We collected whole blood and paper strip samples from mule deer taken during the fall 2002 hunting season. In October

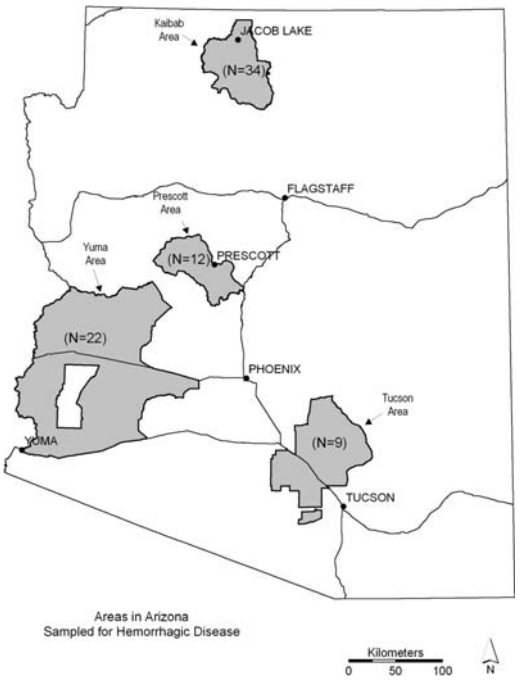

FIGURE 1. Geographic areas in Arizona where hunter-killed male deer were sampled for antibodies to hemorrhagic disease viruses, including the number of paired strip and serum samples taken from each area.

2002, 2,000 sampling kits were sent to individuals with hunt permits for mule deer in four areas (Fig. 1). The Yuma area (33°35′N, 114°0′W) included six Game Management Units (GMUs) in southwestern Arizona, including the Kofa National Wildlife Refuge. The Prescott area (34°30′N, 112°35′W) included three GMUs in central Arizona. The Kaibab area (36°25′N, 112°12.5′W) included two GMUs that encompassed the Kaibab Plateau in northern Arizona, and the Tucson area (32°37.5′N, 111°15′W) included five GMUs in southern Arizona. Each sampling kit contained a 50 ml Falcon centrifuge tube (Becton Dickinson, Franklin Lakes, New Jersey, USA) for blood collection, instructions on how to collect the blood sample, and a list of collection stations for blood samples. Samples were delivered to AGFD personnel and then labeled with the date, GMU, and a unique identification number. Paper strips (1.0×5.0 cm; paper no. 740E, Schleicher and Schuell Inc., Leene, New Hampshire, USA) were completely soaked with whole blood taken from the tubes and then air-dried for approximately 24 hr. Dry strips were stored in empty 50 ml Falcon tubes at room temperature for >1 wk before being shipped overnight to the Southeastern Cooperative Wildlife Disease Study (SCWDS; College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA) for testing. Whole blood tubes were centrifuged in a Dynac model 420101 centrifuge (Becton Dickinson, Sparks, Maryland, USA) at 1,500 × G for 15 min or until serum was easily separated from the clot. Sterile disposable syringes were used to draw off serum. Sera were placed in sterile 50 ml Falcon centrifuge tubes and refrigerated until shipping. Sera were screened for antibodies to BTV and EHDV using agar gel immunodiffusion tests (AGID; Pearson and Jochim, 1979). Because significant cross-reactions can occur with these tests, we interpreted positive results as evidence of previous infection with EHDV and/or BTV (Stallknecht et al., 1991a). To identify EHDV and BTV serotypes, AGID-positive sera were tested by serum neutralization (SN) as described (Stallknecht et al., 1991b). Strip samples were tested by SN only as described (Stallknecht and Davidson, 1992). All testing was completed within 2 mo of blood collection. We used a chi-square test for proportions to test the null hypothesis of no difference in antibody prevalence among areas and considered the result statistically significant at $P<0.05$ (Zar, 1999).

Hunters returned 125 serum samples to AGFD personnel; paired paper strips were taken from 77. A positive serum sample was defined as a positive or weak positive result on either the EHDV or BTV AGID tests with subsequent confirmation by a positive SN test result at a dilution ≥1:10 for at least one EHDV or BTV serotype. A positive for a blood strip

TABLE 1. Positive serum samples (no., %) for hemorrhagic disease viruses by serotype and titer in 42 hunter-killed mule deer in Arizona.

Serotype	Serum neutralizing antibody titer							Total positive for serotype at >1:10[a]
	0	10	20	40	80	160	320	
EHDV 1	7 (17)	4 (10)	2 (5)	2 (5)	5 (12)	5 (12)	17 (40)	35 (83%)
EHDV 2	4 (10)	1 (2)	2 (5)	3 (7)	4 (10)	4 (10)	24 (57)	38 (90%)
BTV 2	38 (90)	2 (5)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	4 (9%)
BTV 10	23 (55)	1 (2)	1 (2)	1 (2)	4 (10)	3 (7)	9 (21)	19 (45%)
BTV 11	16 (38)	1 (2)	1 (2)	5 (12)	4 (10)	5 (12)	10 (24)	26 (62%)
BTV 13	31 (74)	2 (5)	7 (17)	1 (2)	1 (2)	0 (0)	0 (0)	11 (26%)
BTV 17	29 (69)	9 (21)	3 (7)	0 (0)	1 (2)	0 (0)	0 (0)	13 (31%)

[a] Percentage of total AGID and serum SN samples.

was defined as a positive SN test result (1:2 dilution of eluted blood) for at least one EHDV or BTV serotype. Strip and serum samples showed consistent results in 72 of 77 (94%) samples. Thirty-one serum and strip samples showed antibodies against HD viruses. Four negative serum samples were positive on strips, and one positive serum sample had a negative paper strip. Forty-one samples were negative for antibodies on both strip and serum. Prevalence estimates were 46% as determined by blood strip analysis and 42% from serum.

Because we did not receive both strips and serum from all animals, the number of samples used to validate the strip sampling method was not the same as that used in statewide HD surveillance. A total of 42 (34%) statewide serum samples tested positive for antibodies to EHDV and/or BTV. We found considerable variability in presence of antibodies among samples from the four regions of the state. All samples from the Kaibab area ($n=68$) were negative, but antibodies were detected in samples from the three other regions. The proportion of positive samples was 79%, 76%, and 65% in the Tucson ($n=14$), Yuma ($n=29$), and Prescott ($n=14$) areas, respectively. These differences were significant ($\chi^2=76.22$, 3 df, $P<0.00001$).

Antibodies were detected against all of the North American EHDV and BTV serotypes (Table 1). Because cross-reactions can occur in SN tests between serotypes within BTV and EHDV serotypes, evidence of previous exposure to a specific serotype was determined by the presence of clusters (≥50% of all positive samples testing positive to a given serotype) or monospecific test results (testing positive to one serotype within a serogroup at a ≥1:20 dilution) (Stallknecht et al., 1991b, 1995). Both of these criteria were met for EHDV-1, EHDV-2, and BTV-11. Monospecific antibodies also were detected for BTV-10.

Results obtained from dried blood on paper strips proved to be a reliable method to screen a population for antibodies to EHDV and BTV. The strip method makes collection of blood from dead animals much simpler, and the samples do not need to be refrigerated before analyses. However, serum testing should be the preferred method when possible.

Mule deer from the Tucson, Prescott, and Yuma areas were exposed to both EHDV and BTV, but antibodies were not detected in deer from the Kaibab area. Such differences could be related to the distribution of vectors for HD viruses. Unfortunately, the distribution of the *Culicoides* spp. vectors in Arizona is not well known. Recent efforts in the Yuma area (Rosenstock et al., 2003) have found the known vector *C. sonorensis* and a potential new vector (*C. mohave*) to be locally abundant and widely distributed.

Culicoides sonorensis has been collected in the Tucson area (F. Ramberg, University of Arizona, pers. comm.); however, the status of these vectors in the Kaibab and Prescott areas is unknown.

Exposure to HD viruses can vary with elevation or physiographic region (Dubay et al., 2004; Stallknecht et al., 1991a) as well. Among the four sites, elevation ranged from approximately 706 m in Tucson area to 2,621 m in Kaibab area. The Yuma area is at 784 m, and the Prescott area is at 1,450 m. Dubay et al. (2004) identified elevation trends in EHDV/BTV antibody prevalence for mule deer harvested near Prescott. Deer from lower elevations were more likely to be infected than those harvested from higher elevations. Therefore, elevation differences among sites also could explain statewide patterns in HD exposure among mule deer populations.

Observed variation in herd immunity within Arizona may aid the understanding of HD distribution and risk. To date, evidence of HD in Arizona is limited. The first documented cases of HD in free-ranging deer in Arizona occurred in the Tucson area in 1993 (Noon et al., 2002a). Two mule deer also died of HD in Prescott in 2001 (Dubay et al., 2004), but no other carcasses were identified with HD during the same period. Both Tucson and Prescott, Arizona, were identified in this investigation as areas with higher seroprevalence for EHDV and/or BTV. Mule deer from the Kaibab area could be more susceptible to a large-scale HD epizootic than deer from other sites in Arizona because Kaibab deer do not have preexisting antibodies against BTV or EHDV. Gaydos et al. (2002) identified genetic differences in HD susceptibility in subspecies of white-tailed deer experimentally exposed to EHDV viruses: white-tailed deer fawns from Texas (USA) showed mild symptoms of infection, but fawns from Pennsylvania (USA) died after exposure to the virus. If similar innate resistance and susceptibility occurs in mule deer, perhaps animals from the Kaibab area evolved without exposure to HD viruses, and therefore deer from the Kaibab area could be genetically more susceptible to HD viruses. This is of particular concern because the deer population in the Kaibab area is highly prized for trophy buck hunting opportunities. Further research to identify potential causes of variation in HD exposure and susceptibility are warranted.

Dubay et al. (2004) analyzed serum samples from mule deer harvested near Prescott for antibodies against HD viruses. They found serologic evidence of previous exposure to EHDV-1, EHDV-2, BTV-10, BTV-11, and BTV-13. In the current study, evidence of previous infection with EHDV-1, EHDV-2, BTV-10, and BTV-11 was detected. Detection of multiple serotypes of both EHDV and BTV is common in deer populations (Stallknecht et al., 1991b, 1995). In this study, EHDV-2 was the predominant serotype, being present in 90% of positive samples, which is consistent with serologic results from white-tailed deer (Stallknecht et al., 2002).

The presence of BTV and EHDV in deer habitat, and the variation of HD prevalence in mule deer populations, has implications for the translocation of wild ungulates. Given that mule deer from various sites in Arizona showed different levels of exposure to HD viruses, translocations over short distances could be impacted by regional disease differences. Moreover, translocating mule deer that are genetically susceptible to HD viruses to an area where populations are resistant to infection could change the genetic composition of animals in the translocation site, rendering them more susceptible to disease. We recommend monitoring of HD exposure among recipient and source populations to reduce potential impacts to immunologically naive or genetically susceptible animals.

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