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EPIZOOTIOLOGY OF AN EPIZOOTIC HEMORRHAGIC DISEASE OUTBREAK IN WEST VIRGINIA

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ABSTRACT: An outbreak of epizootic hemorrhagic disease virus, serotype 2 (EHDV-2) was responsible for localized white-tailed deer (*Odocoileus virginianus*) mortality in Hardy and Hampshire counties, West Virginia (USA), in the summer and fall of 1993. Using available historical data on regional herd immunity, data opportunistically collected during the epizootic, and postepizootic sampling of hunter-harvested deer, we grossly estimate certain epidemiologic parameters and compare findings to a hypothesis about hemorrhagic disease outbreaks in the Appalachian Mountains. During the epizootic, 57.9 km^2 were actively searched and 228 dead deer were found. Epizootic hemorrhagic disease virus, serotype 2 was isolated from seven of nine deer sampled in Hardy and Hampshire counties. Preepizootic exposure of deer to EHD viruses was unknown, but available data suggest that it was negligible. The geographic distribution of the outbreak was defined by plotting the locations of dead deer found during the outbreak, as well as the locations of deer harvested by hunters after the outbreak that had antibodies to EHDV-2 on a map sectioned into 16.65 km^2 rectangular sections. Sections that included one or more dead deer or hunter-harvested deer with antibodies to EHDV-2 were included in the defined outbreak area. Postoutbreak sampling revealed monospecific EHDV-2 antibodies in 12% of deer harvested by hunters within the defined outbreak area. Based on the available data and accepting certain assumptions, gross calculations suggest that this outbreak appears to have been isolated and probably killed a high percentage of the deer that were infected. This is consistent with the hypothesis that sporadic hemorrhagic disease outbreaks in the Appalachian Mountains are usually localized and severe.

Key words: EHD, epizootic, epizootic hemorrhagic disease, hemorrhagic disease, *Odocoileus virginianus,* West Virginia, white-tailed deer.

INTRODUCTION

Hemorrhagic disease (HD), the most important infectious disease of white-tailed deer (*Odocoileus virginianus*), is caused by orbiviruses (*Reoviridae*) in the epizootic hemorrhagic disease (EHD) virus or bluetongue (BLU) virus serogroups (Nettles and Stallknecht, 1992). Of the two serogroups, the EHD viruses are most often associated with infection in white-tailed deer populations in the southeastern United States (Nettles et al., 1992b). The spatial distribution of HD in the eastern United States is not uniform. Epizootics in northern latitudes are infrequent and are characterized by severe clinical disease and mortality, whereas virus exposure in southern latitudes is more frequent and often results in mild or inapparent disease (Davidson and Doster, 1997). Geographic variations in HD distribution are believed to be attributable to an interaction of various factors including abundance and distribution of competent *Culicoides* midge vectors, the serotype and pathogenicity of EHD and BLU viruses present, levels of existing herd immunity, and genetic variations in deer susceptibility (Stallknecht et al., 2002).

Hemorrhagic disease epizootics are sporadic events in deer in the Appalachian Mountains, including West Virginia (USA),

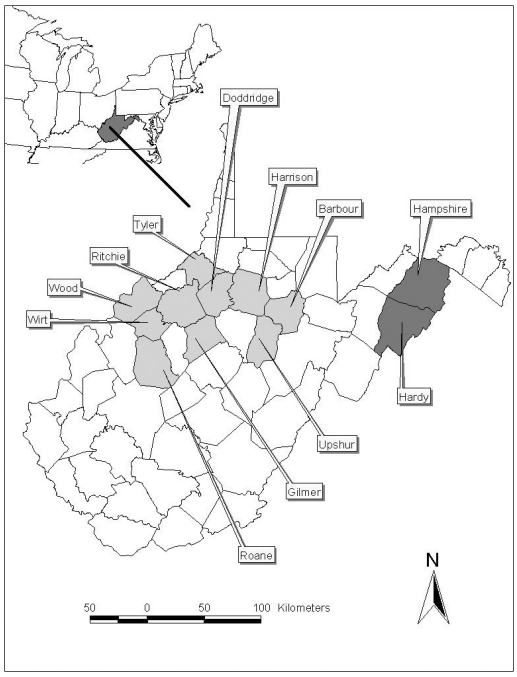

FIGURE 1. Map showing Hardy and Hampshire counties in West Virginia (USA) and locations of counties where HD was reported in West Virginia prior to 1993.

and plateau regions immediately west of the Appalachians (Davidson and Doster, 1997). Based on a questionnaire sent to state wildlife agencies, clinical HD was not reported in West Virginia during 1980, although prior serologic evidence indicated that both EHD and BLU viruses had previously been present (Couvillion et al., 1981). The first documented HD epizootic in West Virginia occurred in 1981 when EHD virus, serotype 2 (EHDV-2) was isolated from dead white-tailed deer in Ritchie County (Nettles et al., 1992a), and HD activity was reported in four other counties (Doddridge, Gilmer, Roane, and Tyler) (Southeastern Cooperative Wildlife Disease Study [SCWDS], unpubl. data) (Fig. 1). The next documented epizootic occurred in 1988 when at least 70 deer died in six counties (Barbour, Harrison, Roane, Upshur, Wood, and Wirt) (Nettles and Stallknecht, 1992) (Fig. 1). In this event, EHDV-2 was isolated from deer from Barbour and Wood counties (SCWDS, unpubl. data). Using reporting standards and annual surveys of state wildlife agencies previously described (Nettles et al., 1992a), HD activity was not reported in West Virginia from 1990 through 1992 (SCWDS, unpubl. data). In the late summer and fall of 1993, a HD outbreak killed numerous white-tailed deer in Hardy and Hampshire counties (Fig. 1), West Virginia. In addition to deer, six cattle herds in these counties exhibited signs of a bluetongue-like disease during this epizootic and were briefly under federal quarantine (J. Plumley, pers. comm.). Cattle presented with weight loss, anorexia, and oral vesicular lesions, but sheep were not involved (J. Plumley, pers. comm.). Although virus isolation attempts performed on whole blood at the National Veterinary Services Laboratory (Ames, Iowa) failed to isolate EHD or BLU viruses, some of the cattle tested did have precipitating antibodies to EHD and BLU viruses, and several animals had serum neutralizing antibodies to EHDV-2 (Thomas, 1993).

Owing to the virtual impossibility of predicting the time and location of a HD epizootic, it is extremely difficult to collect location-specific, preepizootic data and then quickly mobilize the manpower and money needed to investigate an outbreak once it occurs. Consequently, there is little published information describing the direct impacts of HD epizootics on white-tailed deer populations. We attempt to grossly estimate certain epidemiologic parameters for the 1993 HD outbreak in West Virginia by using available historical data on regional herd immunity, data opportunistically collected during the epizootic, and postepizootic sampling of hunter-harvested deer. Also, we try to evaluate the population impact of this epizootic and compare findings to theories about the epidemiology of HD in areas like West Virginia.

MATERIALS AND METHODS

Unusual deer mortality in Hardy and Hampshire counties, West Virginia, was first reported

to the West Virginia Division of Natural Resources (WV DNR) on 11 July 1993 and ended after the first week of October 1993 when ambient temperatures in the area dropped below 0 C. Although dedicated resources were not available to search for dead deer in a thorough and systematic manner, local media coverage alerted private citizens of the outbreak and encouraged them to report dead deer to WV DNR. Personnel from WV DNR recorded the location of each reported deer and actively searched for additional sick or dead deer at reported sites when personnel were available. Except for deer that were in good condition and necropsied, records were only kept on the geographic distribution of dead deer found and not on the temporal distribution of cases. A clinical case was defined as any dead deer reported to or found by WV DNR personnel, where trauma or other cause of death was not immediately obvious. This epizootic occurred prior to widespread use of global positioning systems, so clinical cases were plotted using a map divided into 16.65 km^2 rectangular sections.

One of the authors (J.M.C.) performed field necropsies on 10 dead deer that were in good postmortem condition and recorded gross findings. Six of these deer were found in Hardy County, three were found in Hampshire County, and one deer came from Grant County, located directly west of Hardy County. Spleen, lymph node, and ethylenediaminetetraacetic acid anticoagulated whole blood samples were collected from all 10 deer and were submitted to SCWDS (Athens, Georgia, USA) for virus isolation. Virus isolations were performed as previously described (Quist et al., 1997). Isolates were identified by virus neutralization against all known North American EHD and BLU virus serotypes (Quist et al., 1997).

In West Virginia, hunters are required by law to register harvested deer at game checking stations. Personnel from the WV DNR, assisted by students from West Virginia University, examined 292 hunter-harvested deer brought to eight game checking stations in Hardy and Hampshire counties on 22 November 1993. Age was estimated for most deer using tooth eruption and wear (Severinghaus, 1949), and all deer were examined for signs of chronic HD infection, specifically signs of interrupted hoof growth (Couvillion et al., 1981) on two or more feet. Using 0.5 cm per month as an average rate of hoof growth (Miller et al., 1986), the distance between lesions and the coronary band was used to backdate the occurrence of the disease-causing hoof lesions.

Blood samples were collected from 276 of the 292 hunter-harvested deer and examined using techniques that yield similar results (Stallknecht and Davidson, 1992). Blood samples from 188 of the 276 deer were absorbed onto filter paper strips and dried for later testing. Of the 188 samples collected, 177 were suitable for testing for EHD virus and for BLU virus serum-neutralizing antibodies and were tested as previously described (Stallknecht and Davidson, 1992). Using blood tubes provided by WV DNR personnel, hunters collected serum samples from the other 88 deer while field-dressing deer. Serum from these samples was tested for EHD virus and BLU virus group antigen specific antibodies using agar-gel immunodiffusion (AGID) test kits (Veterinary Diagnostic Technology, Inc., Wheatridge, Colorado, USA) as described by the manufacturer. All positive samples were tested via serum neutralization against all known North American EHD and BLU virus serotypes as previously described (Stallknecht et al., 1995). Seropositive and seronegative hunter-harvested deer were plotted on the same map used to plot locations of dead deer.

The map disclosing the locations of dead and hunter-harvested seropositive and seronegative deer was used to define the geographic distribution of the outbreak. An entire 16.65 km^2 rectangular section was included as part of the outbreak area if one or more dead deer or seropositive hunter-harvested deer was found within the area.

To further help define the geographic distribution of the outbreak, deer from surrounding counties also were examined. Serum samples from 31 hunter-harvested deer from neighboring West Virginia counties located directly west (Grant and Pendleton) and south (Pocahontas) of Hampshire and Hardy counties (Fig. 1) were tested for EHD and BLU virus antibodies. Serum samples from 15 deer were collected in northwest Shenandoah and southwest Frederick counties in Virginia (USA), adjacent to and east of Hardy and Hampshire counties, West Virginia, respectively. These also were tested for EHD virus and BLU virus antibodies. Also, personnel from the Virginia Department of Game and Inland Fisheries Deer Project examined more than 2,000 hunter-harvested deer in adjacent counties to the east (northwestern Virginia).

Successful hunters were interviewed at game checking stations to determine the location from which deer were harvested and to assess the hunter's impression of deer abundance relative to the previous year. Deer harvest sites reported by hunters were plotted into the same 16.65 km^2 rectangular sections used to map locations of dead deer and define the outbreak area. Only results from hunters that reported hunting in the same area in 1992 as they did

TABLE 1. Formulas and calculated epizootic parameters from the hemorrhagic disease outbreak.

Parameter	Formula used	Result
Dead deer per square kilometer searched	[Number of dead deer found (228)] ÷ [area searched (57.9 km^2)]	3.9 dead deer/km^2
Estimated total number of deer that died of EHDV-2[a] in outbreak area	[Outbreak area (499.5 km^2)] × (dead deer per square kilometer searched)	1,948 deer
Estimated deer population at risk in outbreak area	[Outbreak area (499.5 km^2)] × [1992 population estimate (20 deer/km^2)]	9,990 deer
Estimated postepizootic deer population in outbreak area	(Estimated deer population at risk in outbreak area) − (estimated total of deer that died of EHDV-2 in outbreak area)	8,042 deer
Estimated number of deer infected with EHDV-2 that survived	(Estimated postepizootic deer population in outbreak area) × [percentage of hunter harvested deer within outbreak area with antibodies to EHDV-2 (12%)]	965 deer
Estimated total number of deer infected with EHDV-2 during epizootic	(Estimated total of deer that died of EHDV-2 in outbreak area) + (estimated number of deer infected with EHDV-2 that survived)	2,913 deer
EHDV-2 infection rate	(Estimated total number of deer infected with EHDV-2 during epizootic) ÷ (estimated deer population at risk in outbreak area)	29%
Mortality rate	(Estimated total of deer that died of EHDV-2 in outbreak area) ÷ (estimated deer population at risk in outbreak area)	20%
Case fatality rate	(Estimated total of deer that died of EHDV-2 in outbreak area) ÷ (estimated total number of deer infected with EHDV-2 during epizootic)	67%

[a] EHDV-2 = epizootic hemorrhagic disease virus, serotype 2.

in 1993 were used to compare hunter impression of deer abundance inside and outside of the outbreak area relative to the previous year. Results from the hunter interview were compared by Wilcoxon/Kruskal-Wallis one-way analysis of variance (Sall and Lehman, 1996).

Deer densities in Hardy and Hampshire counties for years 1990 through 1995 were estimated using county-specific annual antlered buck harvest for a buck-only, 2-wk hunting season, which has been in effect in these counties since 1961. Hunters are required by law to register all harvested deer at deer checking stations in West Virginia, and the county-specific harvest was recorded and used to calculate buck kill per square mile (excluding urban-industrial areas, streams, reservoirs, and highways). Annual harvest was then converted to a rough population estimation of the fall prehunt population as per Harlow and Jones (1965).

Epidemiologic parameters were calculated using standard definitions (Toma et al., 1999), while accepting certain assumptions. We assumed all white-tailed deer carcasses found during the outbreak died of EHDV-2 infection, except where trauma or other cause of death was immediately obvious. Assuming uniform distributions of deer and virus activity, the number of dead deer found per square kilometer searched was extrapolated over the entire outbreak area to estimate total deer mortality due to EHDV-2 within the outbreak area (Table 1). We also assumed that all deer present were naive to EHD viruses and equally susceptible to infection. Under this assumption, we estimated the susceptible deer population within the outbreak area by multiplying the 1992 estimated deer density for Hardy County, where most mortality occurred, by the total outbreak area (Table 1). We assumed that mortality other than EHDV-2 was insignificant during the epizootic and calculated the estimated postepizootic deer population by subtracting the estimated total number of deer that died of EHDV-2 from the starting estimated population (Table 1). We assumed that hunter-harvested deer were representative of the deer population after the EHDV-2 outbreak and used the percentage of hunter-harvested deer within the outbreak area with antibodies to EHDV-2 to calculate the number of deer that were exposed to EHDV-2 and survived infection (Table 1).

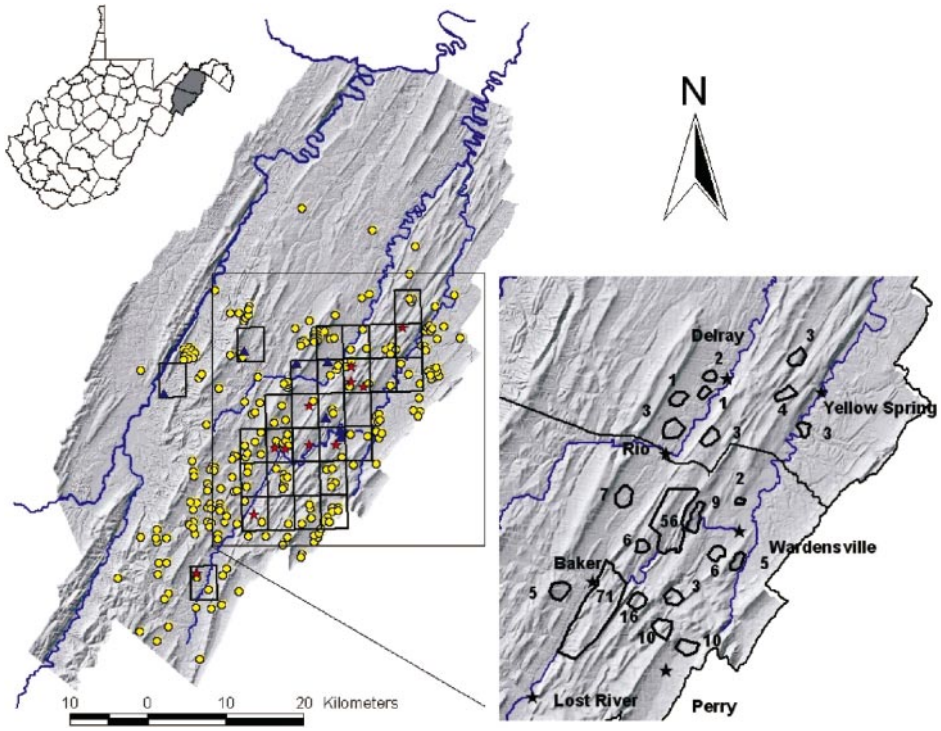

FIGURE 2. Maps of Hardy and Hampshire counties, West Virginia, illustrating major rivers, county boundaries, and the locations of deer from which epizootic hemorrhagic disease virus, serotype 2 was isolated (▲) (n=7), hunter-harvested seronegative deer (○) (n=265), and hunter-harvested seropositive deer (★) (n=11; note, the harvest location of one seropositive deer was unknown). The inset map depicts locations and dimensions of areas (n=21) actively searched for dead deer, the number of dead deer found in those areas, and the seven towns roughly surrounding the outbreak area.

RESULTS

A total of 228 dead deer were found (Fig. 2), where trauma or other cause of death was not immediately obvious. Of those, 207 were in Hardy County with the remaining 21 in bordering northern Hampshire County. In most instances, dead deer were found along watercourses, especially small feeder streams. Although the total area that was searched was approximately 57.9 km^2, the defined outbreak area encompassed 30 of the 16.65 km^2 rectangles (Fig. 2), for a total estimated area of 499.5 km^2. The outbreak area was roughly bounded by the West Virginia towns of Baker, Rio, Delray, Yellow Spring, Wardensville, Perry, and Lost River (Fig. 2).

Seven of nine deer necropsied from Hardy and Hampshire Counties had gross lesions that were consistent with hemorrhagic disease, including erosions of the dental pad, pulmonary edema, and hemorrhage on serosal surfaces of the gastrointestinal tract. Epizootic hemorrhagic disease virus, serotype-2 was isolated from all seven deer that exhibited gross lesions consistent with hemorrhagic disease (Fig. 2). Positive deer ranged in age from 3-mo to 9-yr old with most isolations (n=4) coming from deer within the 1.5- to 4.5-yr-old age classes. Dead deer that exhibited gross lesions consistent with HD and were positive for virus isolation were found on 23 August, 4 September (two animals), 13 September, 20 September, 27 September, and 7 October 1993. Gross lesions consistent with hemorrhagic disease were not apparent in two of the deer found dead in Hardy County (29 July and 12 September

1993) and necropsied. Virus isolation attempts on tissues and blood from these two deer failed to isolate any viruses. The one deer found dead in Grant County (14 October) exhibited gross lesions consistent with trauma, and consequently it was not included as a mortality related to the EHDV-2 epizootic. Incidentally, virus isolation attempts on tissues and blood from this animal also failed to isolate any viruses.

Of 292 hunter-harvested deer examined in West Virginia for signs of disease, 4.8% ($n=14$) had hoof lesions estimated to have occurred 2 to 4 mo earlier. Of the 177 filter paper test strip samples suitable for testing, 6% ($n=10$) had monospecific antibodies to EHDV-2 (Fig. 2). Of the 88 serum samples tested, two from Hampshire County had monospecific serum neutralizing antibody titers to EHDV-2 (Fig. 2). Antibodies to EHDV-2 were detected in deer from the 1.5-, 2.5-, and 3.5-yr-old age classes, but not from the 0.5- and 4.5-yr-old age classes, of which only one and six deer, respectively, were tested. Of the 102 deer harvested by hunters within the defined 499.5 km^2 outbreak area, 12% ($n=12$) had antibodies to EHDV-2. Of the 14 deer with hoof lesions, only 11 animals had samples collected that were suitable for testing for antibodies. Nine of these 11 animals (82%) had monospecific antibodies to EHDV-2 as described.

Of 292 successful hunters interviewed (those that came to a checking station with a buck to legally report), 216 reported they had hunted in the same location the previous year. Of hunters who hunted in the same area as they did the previous year, 61% (55/90) of those that hunted within the defined outbreak area reported seeing slightly or significantly fewer deer compared with 41% (52/126) who hunted outside of the outbreak area. The difference was not significant ($P=0.07$).

None of the 31 hunter-harvested deer tested from the neighboring West Virginia counties of Grant, Pendleton, and Pocahontas were positive for antibodies to EHD virus or BLU viruses. Of the 2,000 hunter-harvested deer in northwest Virginia, hoof lesions or other signs of chronic HD infection were not apparent in any deer. Of the 15 deer sampled in northwest Shenandoah and southwest Frederick counties, Virginia, none had antibodies to EHD or BLU viruses.

Deer density in Hardy County, West Virginia, during the 1990 hunting season was 15/km^2. For the subsequent years 1991 through 1995, deer density was estimated to be 19/km^2, 20/km^2, 14/km^2, 15/km^2, and 19/km^2, respectively.

The susceptible deer population within the outbreak area was estimated to be 9,990 animals (Table 1). Total deer mortality due to EHDV-2 was estimated to be 1,948, and we estimated that 965 deer were exposed to EHDV-2 and survived (Table 1). From these estimates, we calculated an EHDV-2 infection rate of 29%, a mortality rate of 20%, and a case fatality rate of 67% (Table 1).

DISCUSSION

Based on the season of the outbreak, gross lesions characteristic of HD, isolation of EHDV-2, and monospecific EHDV-2 antibodies present in deer harvested by hunters after the epizootic, we believe this outbreak was caused exclusively by EHDV-2. The fact that nine of 11 deer with hoof lesions also tested positive for monospecific antibodies to EHDV-2 supports that the hoof lesions identified in 14% of hunter-harvested deer resulted from disease caused by prior infection EHDV-2. Although a serologic survey for HD antibodies was not performed in this white-tailed deer population just before the EHDV-2 outbreak, this population was presumed to be naive. This is because, despite surveillance, hemorrhagic disease activity has never been reported from either of these two counties or from adjacent counties in Virginia or West Virginia since annual reporting began in 1980 (Nettles et al., 1992a; SCWDS, unpubl. data) (Fig. 1).

This does not discount that isolated EHD activity could have occurred in these counties without notice, but it does support that herd immunity to EHD viruses was most likely negligible. Although it does not confirm the absence of antibodies to HD viruses in white-tailed deer from this area, random sampling and testing for antibodies to HD viruses in deer from this area have been uniformly negative. Of five deer per year randomly sampled from one site in Hardy County in 1981, 1984, and 1989 as part of deer herd health monitoring, antibodies to EHD and BLU viruses were not detected (SCWDS, unpubl. data). Similarly, in neighboring Mineral County, none of five deer sampled during a herd health check in 1989 had antibodies to EHD or BLU viruses.

Deer mortality was the primary method for determining the distribution of this EHD epizootic. Although dedicated resources were not available and search efforts to locate dead deer were neither systematic nor comprehensive, a considerable effort was put forth by private citizens and WV DNR personnel to locate and map dead deer. This said, only a small percentage of deer that were estimated to have died during the outbreak were found. In order to more definitively designate the distribution of the epizootic, it would have been ideal to have had the resources and personnel to mount a thorough search and recovery effort throughout Hardy and Hampshire counties, as well as in adjacent counties over the course of the outbreak. Since this was not possible, it is difficult to definitively state that the outbreak did not extend beyond the 30 identified 16.65 km^2 rectangular sections (Fig. 2). Media attention focused on the outbreak was strong, however, and we suspect that with the exception of isolated deaths, farmers, hikers, and private landowners would have found and reported larger clusters of mortality. The one deer found dead in Grant County, West Virginia, and reported to WV DNR clearly died of trauma, but the fact that the public recognized even the remote possibility that this deer could have been a part of the Hardy and Hampshire County EHDV-2 epizootic is a testament to the widespread awareness of the epizootic in counties surrounding Hardy and Hampshire. Combined, these facts suggest that although far from exact, deer mortality is probably a strong foundation for defining the outbreak area for the purposes of grossly estimating the epizootic impact on this population.

Accepting the tenable premise that deer in this area had not previously been exposed to EHD viruses, serology performed on hunter-harvested deer also was used to help delineate the outbreak area. The negative serologic results from 46 hunter-harvested deer from three neighboring West Virginia counties and two bordering Virginia counties support the idea but do not confirm that this epizootic was isolated to Hardy and Hampshire counties. Assuming a high case fatality rate would occur throughout the region and that consequently only a small percentage of deer infected would survive and have antibodies to EHDV-2, a larger sample size is probably necessary to definitively argue that the outbreak was confined to these two counties based on the absence of antibodies to EHD viruses alone. Given that 4.8% of the hunter-harvested deer examined in Hardy and Hampshire counties had hoof lesions estimated to have occurred 2 to 4 mo earlier, the lack of signs of chronic HD in more than 2,000 hunter-harvested deer in adjacent northwest Virginia more strongly supports the idea that an HD epizootic did not extend into this region.

Within Hardy and Hampshire counties, our ability to delineate the outbreak area using serology was limited by opportunistic sampling of hunter-harvested deer. Of the 265 hunter-harvested deer sampled, 163 came from outside of what was defined as the outbreak area, representing 54 of the 16.65 km^2 rectangular sections used to map the outbreak. With the exception of four of the 30 rectangular sections defined as the outbreak area, these 54 sec-

tions completely encompass the defined outbreak area. While additional samples would have been ideal to help conclusively prove that the real outbreak did not extend beyond the defined area, data from serologically negative hunter-harvested deer support that the defined area probably reasonably approximates the real outbreak area.

The origin of the virus circulating in this epizootic is unknown. Lack of evidence of recent HD outbreaks in West Virginia combined with a history of only rarely detecting antibodies to EHD and BLU viruses in sampled West Virginia deer, however, suggest that it is highly unlikely that the virus originated within the affected area in West Virginia. Other work conducted supports the suggestion that HD is not considered enzootic in these areas and that antibodies to HD viruses in white-tailed deer from mountain physiographic provinces are rare, especially in more northerly latitudes (Stallknecht et al., 1991a). In areas where HD viruses are thought to continually circulate within populations, antibody prevalence within deer herds can be as high as 100% (Stallknecht et al., 2002). A recent phylogenetic analysis of EHDV-2 (Murphy, 2003) suggests that the virus from the West Virginia epizootic likely arose from a distinct virus population not closely related to other North American epizootics. Three of the 1993 West Virginia EHDV-2 isolates from this study were included in phylogenetic analyses of over 74 EHDV-2 North American isolates recovered from 1962 to 2001. Neighbor-joining analyses of both the S10 and L2 gene segments, as well as a statistical parsimony network analysis of the L2 segment, demonstrated that the West Virginia isolates formed a distinct subclade, separate from the other isolates.

Despite EHDV-2 not being isolated from cattle in quarantined herds, precipitating antibodies to EHD and BLU viruses, as well as serum neutralizing antibodies to EHDV-2 identified in several animals, suggest that cattle also could have been involved in this EHDV-2 epizootic. Although infection with EHDV-2 occasionally causes disease in adult cattle (Metcalf et al., 1992), calves infected with EHDV-2 developed viremia but did not develop detectable clinical disease (Abdy et al., 1999). Experimental infection of calves with EHDV-2 demonstrated that calves can remain viremic for up to 44 days (Abdy et al., 1999). It is possible that calves moved into Hardy or Hampshire county in 1993 may have been asymptomatically infected with EHDV-2, especially if they were moved from more southern latitudes such as south Florida, where EHD viruses are considered to be endemic. Alternatively, it is possible that EHDV-2 infected *Culicoides* were carried into West Virginia by wind, which has been hypothesized previously as a mechanism for introducing HD viruses (Sellers and Maarouf, 1991). Experimental infection has shown that white-tailed infected with EHDV-2 can be viremic for up to 61 days (Gaydos et al., 2002). White-tailed deer are nonmigratory, and it would have been virtually impossible for a white-tailed deer infected with EHDV-2 to travel on its own from an EHD-enzootic area north into Hardy or Hampshire counties, West Virginia, suggesting it is highly unlikely that wild white-tailed deer were the source of the virus. Since some white-tailed deer can be asymptomatically infected with EHDV-2 (Gaydos et al., 2002), another consideration for viral origin could have been the unintentional importation of an EHD-infected, yet asymptomatic white-tailed deer from a virus-endemic area or an area experiencing an EHDV-2 epizootic to a pen-rearing white-tailed deer facility in Hardy or Hampshire counties. The presence or absence of pen-raised (farmed) white-tailed deer and the importation of new deer into Hardy and Hampshire counties during the summer of 1993, however, is unknown.

Epizootics of HD are considered sporadic events in West Virginia, and it is thought that when outbreaks do occur

they cause high mortality in an isolated area. Definitive impacts of HD on white-tailed deer herds are difficult to determine, however, without accurate preoutbreak and postoutbreak population estimates and accurate and complete data on mortality during an outbreak (Stallknecht et al., 2002). It is because these data are extremely difficult, if not impossible, to obtain that there is so little information available on the impacts of HD epizootics on white-tailed deer populations. This information, however, is important for biologists, who need to account for the impacts of epizootics when managing deer herds. Although the data used and assumptions to grossly estimate certain epidemiologic parameters for this EHD outbreak are not ideal, they represent a best estimate opportunity to approximate epizootic population impacts.

Although a population census is ideal for determining preepizootic and postepizootic populations, it is rarely possible to do this with wildlife. In this case, a population density estimate was the only datum available to approximate the number of deer at risk during the outbreak. To determine the number of deer that died during the outbreak and the remaining number of deer present after the outbreak, we chose to extrapolate the number of deer found over the area searched to the entire outbreak area (Table 1). Assuming uniform distributions of deer and virus activity throughout the outbreak area is not entirely accurate, but we felt it was a more accurate method of estimating mortality and postoutbreak population size (Table 1) than was using a population density estimate based on the 1994 buck harvest. Under the assumptions made, detected deer mortality during the epizootic represented only 12% (228/1,948) of estimated mortality. Although this 12% detection rate is low, it is understandable since resources and manpower were not available for a systematic and thorough search for carcasses. Additionally, a 12% detection rate is consistent with previous findings where a mortality rate dramatically lower than what was estimated for this outbreak would not have been detected during an EHDV-2 outbreak in Missouri if deer had not been radio-monitored (Beringer et al., 2000).

Hunters could have avoided harvesting lame deer, thin deer, or deer with other potential chronic sequela to EHD infection. Because none of the 292 hunters interviewed reported seeing but not harvesting such animals, we felt that hunter bias in selecting deer to harvest was negligible and that it was tenable to use the percentage of hunter harvested deer with antibodies to estimate the number of deer that survived infected with EHD-2. Acknowledging and accepting the set of assumptions made, we are comfortable with the calculated mortality rate of 20% and case fatality rate of 67% for this epizootic. While these numbers are likely not exact, we feel they are plausible and representative of the severity of the epizootic and impact on the population.

A mortality rate close to 20% in a deer herd should be detectable by reviewing annual population densities developed from hunter-harvest data. Based on WV DNR estimates, deer density in Hardy County, West Virginia, where most of the mortality occurred during this epizootic, decreased from 20/km^2 in 1992 (preepizootic) to 14/km^2 in 1993 (postepizootic). Many variables besides disease regulate deer density, but it is interesting to note that the estimated 20% mortality rate is consistent with a 30% decrease in Hardy County deer density from 1992 to 1993.

When surveyed, a higher percentage of hunters who hunted within the defined outbreak area reported seeing slightly or significantly fewer deer in 1993 versus 1992 when compared to hunters who hunted outside of the defined outbreak area. Although not significant ($P=0.07$), and understanding that many variables besides actual deer density can influence hunter opinion about deer density, hunter opinion of seeing fewer deer within the

outbreak area when compared to the year prior is consistent with large deer mortality occurring within the defined outbreak area. Owing to manpower constraints and practicality issues, we were only able to interview successful hunters as they brought deer to checking stations. Should dedicated funding and adequate manpower be available in the future, it may be more accurate to interview nonsuccessful as well as successful hunters when comparing hunter attitudes about deer density within and outside of defined outbreak areas.

Of the 102 deer harvested by hunters within the defined 499.5 km^2 outbreak area, only 12% had antibodies to EHDV-2. There is little information available on postepizootic herd immunity in isolated deer populations with which to compare these results. After a 1980 suspected HD outbreak on Ossabaw Island, a small barrier island off the coast of Georgia, 34% of hunter-harvested deer sampled had antibodies to EHD viruses (Stallknecht et al., 1991b). Annual sampling of the Ossabaw herd after the 1980 epizootic demonstrated that herd immunity disappeared rapidly (Stallknecht et al., 1991b). In the case of the Hardy and Hampshire county deer herds, postoutbreak herd immunity was 35% lower than on Ossabaw Island and, thus, would be expected to disappear even faster. In this region, deer lack innate immunity to HD viruses, and HD viruses do not persist. If epizootics have a high case fatality rate and only a low percentage of deer survive infection and develop antibodies against EHDV-2, postepizootic herd immunity is probably insufficient to prevent epizootics during subsequent viral incursions or even appreciably lessen herd mortality. Consequently, managers can expect that future EHD epizootics in this region will probably continue to have similar population impacts, even if viral incursions become more frequent.

We estimated that the 1993 EHDV-2 epizootic in Hardy and Hampshire counties, West Virginia, probably killed approximately 20% of the deer herd and that somewhere near 67% of the deer infected with EHDV-2 died. While these numbers represent gross estimates and are not exact, they are plausible and we feel they support the hypothesis that HD epizootics in deer in the Appalachian Mountains, including West Virginia, and plateau regions immediately west of the Appalachians are characterized by severe clinical disease and high mortality. Historic surveillance data compiled on HD epizootics in West Virginia support the suggestion that outbreaks are sporadic in this area. Finally, even though it would have been better to have conducted more thorough and systematic search efforts for dead deer and collected a greater number of serum samples from hunter-harvested deer more evenly distributed around the proposed outbreak area, we feel that evidence strongly suggests that this outbreak was relatively isolated.

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