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SHORT COMMUNICATIONS

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Hemorrhagic Disease in Kansas: Enzootic Stability Meets Epizootic Disease

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ABSTRACT: Kansas (USA) could represent a transition area between contrasting epidemiologic patterns of hemorrhagic disease (HD) in the midwestern United States. In this study, we compare the distribution of reported clinical HD with serologic data to determine whether the risk of HD in white-tailed deer (*Odocoileus virginianus*) is associated with geographic location corresponding to the reported distribution of two white-tailed deer subspecies. On the basis of a high prevalence of antibodies (91–100%) to multiple serotypes of epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV), with correspondingly few reports of clinical HD, it appears that a state of enzootic stability exists in central and western Kansas. This area corresponds to the reported range of *O. virginianus texanus.* In contrast, in the eastern third of the state, which corresponds to the reported range of *O. virginianus macrourus*, antibody prevalence is significantly lower (45%), EHDV serotypes appear to predominate, and HD, as confirmed by virus isolation, has been consistently reported. These results suggest an abrupt demarcation between enzootic stability in central and western Kansas to a pattern of epizootic HD within the eastern part of this state. Understanding host, vector, and environmental variables responsible for these contrasting patterns could have application to understanding the risk of HD in the midwestern United States.

Key words: Bluetongue virus, enzootic stability, epidemiology, epizootic hemorrhagic disease virus, hemorrhagic disease, Kansas, *Odocoileus virginianus,* white-tailed deer.

Hemorrhagic disease (HD) in wild ungulates is caused by viruses in the epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) serogroups (*Orbivirus, Reoviridae*). Within these serogroups, only EHDV serotypes 1 and 2 and BTV serotypes 2, 10, 11, 13, and 17 have been documented in North America (Stallknecht et al., 1995). With the exception of BTV-2, all of these viruses have been associated with HD in white-tailed deer (*Odocoileus virginianus*; Howerth et al., 2001).

Clinical response to infection of white-tailed deer with both the EHDV and BTV can range from acute mortality to subclinical and is dependent on geographic location (Nettles and Stallknecht, 1992; Nettles et al., 1992; Davidson and Doster 1997; Stallknecht et al., 2002). In general, the frequency of HD decreases with increasing latitude; however, the severity of the disease and the number of cases that result in mortality increase with increasing latitude. On the basis of clinical reports of HD (Nettles et al., 1992), this trend appears to exist throughout the midwestern United States. In the northern portion of this area, including the states of Nebraska, South Dakota, and North Dakota (USA), HD is most frequently detected in the form of sporadic outbreaks with high mortality (Stallknecht et al., 2002). In contrast, in the southern part of this area that includes the states of Texas and Oklahoma (USA), a pattern of enzootic stability characterized by a high rate of infection with minimal or no clinical disease appears to predominate (Kocan et al., 1982, 1987; Stallknecht et al., 1996).

The observed regional variation in clinical response associated with EHDV and BTV infections probably involves the combined effects of acquired and innate immunity. It has been demonstrated that maternal antibodies to EHDV and BTV can

persist for up to 23 wk of age, potentially protecting fawns during their initial exposure to these viruses (Gaydos et al., 2002c). Acquired immunity through previous infection with a homologous or related serotype of EHDV and BTV also has been demonstrated (Quist et al., 1997; Gaydos et al., 2002b) but is protective only to challenge with a virus within the same serogroup (Hoff and Trainer, 1974). Innate immunity to EHDV-1 and EHDV-2 also has been demonstrated in experimental infections of two subspecies of white-tailed deer (*O. virginianus texanus* vs. *O. virginianus borealis*; Gaydos et al., 2002a). Although both subspecies were infected, extreme differences in disease susceptibility were observed, with mortality rates ranging from 0% (*O. virgninaus texanus*) to 100% (*O. virginianus borealis*).

The reported original range of *O. virginianus texanus*, a subspecies that appears to be resistant to EHDV infection (Gaydos et al., 2002a), extends from Texas through Nebraska (Baker, 1984). The northern range extends into southern South Dakota, where it is replaced by *O. virginianus dakotensis*. The eastern limit, where *O. virginianus texanus* joins *O. virginianus macrourus*, occurs in the eastern third of Nebraska, Kansas, and Oklahoma. Unlike *O. virginianus texanus*, both *O. virginianus dakotensis* and *O. virginianus macrourus* occupy areas that are characterized by sporadic HD epizootics (Nettles and Stallknecht, 1992), suggesting that these animals are highly susceptible to HD.

Kansas could represent a transition area between contrasting patterns of enzootic stability and sporadic HD epizootics. Within this state, two white-tailed deer subspecies with potential differences in HD susceptibility are juxtaposed. The western two thirds of the state is reportedly occupied by *O. virginianus texanus*, whereas *O. virginianus macrourus* is reported in the eastern third (Baker, 1984). Although reports of HD from Kansas (Nettles et al., 1992; Southeastern Cooperative Wildlife Disease Study [SCWDS], unpubl. data) are mostly associated with counties in the eastern part of the state, data on the extent of EHDV or BTV infection in white-tailed deer in Kansas are not available. In this study, we compare the reported distribution of clinical HD with that of serologic data (evidence of infection) to determine whether a pattern of enzootic stability exists in western Kansas and to ascertain whether this pattern corresponds with the reported distribution of a potentially resistant white-tailed deer subspecies (*O. virginianus texanus*).

Serum samples from white-tailed deer and mule deer (*Odocoileus hemionus*) were collected by personnel from the Kansas Department of Wildlife during December 1998, October–December 2001, and January 2002. Additional information regarding the incidence of HD in Kansas from 1989 to 2002 was obtained from an annual survey of state wildlife agencies conducted by the SCWDS as described by Nettles et al. (1992). The criteria used to determine a case of HD included reports of the following: 1) sudden, unexplained high deer mortality during the late summer and early fall; 2) necropsy diagnosis of HD as rendered by a trained wildlife biologist, a diagnostician at a State Diagnostic Laboratory or Veterinary College, or SCWDS personnel; 3) isolation of an EHDV or BTV from a deer; or 4) observation of hunter-killed deer that showed sloughing hooves, ulcers in the mouth, or scars on the rumen lining. Virus isolations from deer reported in this study all were made by SCWDS between 1992 and 2002 from spleen and blood samples submitted by the Kansas Department of Wildlife. Viruses were isolated with cattle pulmonary artery endothelial or baby hamster kidney (BHK_{21}) cell lines (Quist et al., 1997). Serum samples were tested for antibodies to EHDV and BTV as previously described (Stallknecht et al., 1995). Samples were initially screened by agar gel immunodiffusion (AGID) EHDV and BTV tests (Pearson and Jochim, 1979). Samples testing positive on either AGID test were fur-

ther tested by serum neutralization (SN) against all known North American EHDV and BTV serotypes. Because significant cross-reactions can occur with the EHDV and BTV AGID tests, an animal was considered positive if it tested positive on one or both of these tests. Antibody prevalence therefore reflects the proportion of animals seropositive to EHDV, BTV, or both. Evidence of previous exposure to a given EHDV or BTV serotype was determined by detection of monospecific reactions or clusters of seropositive results for a given serotype as described (Taylor et al.,1985; Stallknecht et al., 1995). A monospecific reaction was accepted as evidence of previous exposure to a given serotype only if positive SN results at a serum dilution of 1:20 or higher were limited to one serotype within the EHDV or BTV serogroups. A "cluster" was defined as a serotype in which neutralizing antibodies were detected against that serotype in 50% or more of the AGID-positive samples.

For analysis, the state of Kansas was divided along county lines into three geographic regions (eastern, central, and western) approximately equal in size. The division between the central and eastern regions approximates the reported interface between *O. virginianus texanus* and *O. virginianus macrourus.* The central and western regions were divided so that three areas of approximately equal size could be used to test for longitudinal variation, as has been reported in Texas (Stallknecht et al., 1996). Serotype diversity was determined on a regional basis within Kansas. Differences in antibody prevalence were tested by chi-square or Fisher's exact test (EPI Info, 2000, Centers for Disease Control and Prevention, Atlanta, Georgia). Only white-tailed deer results were used for statistical analyses.

Serum samples were obtained from 87 animals (82 white-tailed deer and five mule deer) from 38 counties (Fig. 1). Statewide, a difference in antibody prevalence was not detected ($P=0.949$) between white-tailed deer sampled in 1998 ($n=33$, 67% seropositive) and 2001–02 ($n=49$, 67% seropositive). Antibody prevalence estimates and serotype diversity by region and species are given in Table 1. Differences in antibody prevalence were detected between regions ($P<0.00001$). Antibody prevalence for the eastern region (45%), approximating the reported range of *O. virginianus macrourus,* also was significantly lower ($P<0.00001$) than the prevalence for the combined central and western regions (92%), approximating the reported range of *O. virginianus texanus.*

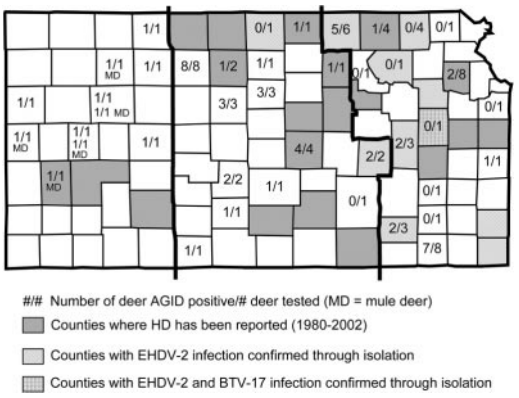

FIGURE 1. Reported hemorrhagic disease and the prevalence of antibodies to epizootic hemorrhagic disease virus (EHDV), bluetongue virus (BTV), or both (detected by agar gel immunodiffusion [AGID]) in white-tailed deer and mule deer in Kansas.

Predominant serotypes, as detected through SN tests, varied by region, but evidence of previous exposure to all EHDV and BTV serotypes, except BTV-2, was observed (Table 1). Epizootic hemorrhagic disease virus–2 was the only serotype detected in all regions and represented the predominant virus isolated from white-tailed deer. Of the 18 viruses isolated from white-tailed deer from Kansas (1994–2002), 17 (94%) were EHDV-2 and one was identified as BTV-17. Evidence of previous BTV infection, as estimated from the percentage of AGID-positive animals with neutralizing antibodies to one or more BTV serotypes, increased in a westerly direction.

Most of the counties in Kansas from which HD was reported (1980–2002) were

TABLE 1. Prevalence of antibodies and serotype diversity of epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) in white-tailed deer (WTD) and mule deer (MD) and reports of hemorrhagic disease (HD) from Kansas.

Area	Species	No. tested	No. positive by AGID (%)[a]	No. tested by SN[b]	Percent positive[c] EHDV SN	Percent positive[c] BTV SN	Serotypes[d] found	Counties reporting HD[e] (%)
East	WTD	44	20 (45)[1]	18	18 (100)	4 (22)	EHDV-1, -2	15/36 (42)[1]
Central	WTD	32	29 (91)[2]	28	26 (93)	18 (64)	EHDV-1, -2 BTV-10, -11, -13, -17	13/37 (35)[1]
West	WTD	6	6 (100)[2]	6	5 (83)	5 (83)	EHDV-2 BTV-10, -11	
West	MD	5	5 (100)	5	5 (100)	3 (60)	EHDV-2 BTV-11	3/31 (10)[2]

[a] Positive on EHDV or BTV agar gel immunodiffusion (AGID) or both. Regional prevalence estimates (with different numbers) are significantly different at $P<0.05$.

[b] Number of AGID positives tested by serum neutralization (SN).

[c] Percentage of positive AGID samples testing positive (1:10 dilution) to one or more EHDV or BTV serotypes.

[d] Serotypes as detected by monospecific reactions or clusters of seropositive results.

[e] Number of counties in which HD was reported in 1980–2002/total counties (percent positive). Proportions of counties reporting HD for region (with different numbers) are significantly different at $P<0.05$.

located in the eastern part of the state, and a significant difference ($P=0.0115$) was detected between the proportions of counties reporting HD by area (Table 1). With one exception, all of the counties ($n=11$) where HD was confirmed through virus isolation were located in the eastern area.

Results suggest that a state of enzootic stability exists in central and western Kansas approximately corresponding to the reported range of *O. virginianus texanus.* In Texas, enzootic stability is characterized by a high antibody prevalence to EHDV and BTV that approaches 100%, by exposure to multiple serotypes of EHDV and BTV with serotype diversity increasing in a westerly direction, and by an inverse relationship between reported HD and observed antibody prevalence (Stallknecht et al., 1996). All of these characteristics were observed in the Kansas deer population.

Although the east to west variation in antibody prevalence and reported HD observed in Kansas represents a similar pattern to that reported for Texas (Stallknecht et al., 1996), this system is unique in that the area of apparent enzootic stability immediately borders an area characterized by sporadic epizootics. Epizootic areas for HD in the southeastern United States are characterized by a low to moderate antibody prevalence, limited serotype diversity, and the observation of mortality associated with infection (Stallknecht et al., 2002). In addition, EHDV-2 usually appears as the predominant serotype present in such areas. All of these characteristics are present in eastern Kansas. This variation suggests an abrupt transition between enzootic and epizootic patterns of HD in this state, and this division appears to correlate with the reported ranges of white-tailed deer subspecies. This pattern is consistent with observations from bordering states; sporadic HD patterns characterized by mortality have predominated in Nebraska and Missouri (USA), with a relative absence of reported HD in Colorado (USA) and Oklahoma (Nettles et al., 1992; Fischer et al., 1995; Nettles, unpubl. data). Kansas, therefore, might represent the northern and eastern limits of enzootic stability in the midwestern United States.

Variation in resistance to clinical HD in white-tailed deer has been detected in experimental studies at the subspecies level (Gaydos et al., 2002a). Field results from Texas (Stallknecht et al., 1996) and Oklahoma (Kocan et al., 1982, 1987) and results from this study also support the hy-

pothesis that *O. virginianus texanus* is highly resistant to HD. However, care must be taken in extrapolating from these results because such resistance might be only indirectly or partially related to subspecies. For example, *O. virginianus texanus* appears to be vulnerable to HD in its northern range in Nebraska and South Dakota, where white-tailed deer mortality associated with HD is commonly reported. On the basis of this discrepancy, it is possible that resistance to HD is spatially restricted to populations under a constant selective pressure related to annual or potential year round transmission of EHDV and BTV. Such areas might be restricted within the range of *O. virginianus texanus.* In addition, considerable change in reported subspecies distributions could have occurred as a result of white-tailed deer reestablishment through restocking efforts. White-tailed deer subspecies distributions used in this study (Baker, 1984) also were morphologically rather than genetically based. Although there is no indication that significant introductions of white-tailed deer occurred in Kansas (McDonald and Miller, 1993), specific genetic information on these populations and subspecies are needed before reliable inferences related to susceptibility can be made.

Results from this and other studies suggest that innate resistance to HD can evolve in areas where deer are continuously challenged with EHDV and BTV. Western Kansas appears to be such an area, but within this state, there appears to be an abrupt transition between areas of enzootic stability and epizootic HD. Understanding the host and environmental factors responsible for this demarcation boundary has application to evaluating risk associated with the movement and introduction of deer and other wild ungulates. In addition, Kansas might represent an ideal location for future field studies related to potential genetic and environmental risk factors associated with HD in the midwestern United States.

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