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Epizootic Hemorrhagic Disease Virus and Bluetongue Virus Seroprevalence in Wild White-tailed Deer (*Odocoileus virginianus*) in Florida, USA

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ABSTRACT: A wild population of white-tailed deer (*Odocoileus virginianus*) was surveyed for evidence of past or current epizootic hemorrhagic disease virus (EHDV) and current bluetongue virus (BTV) infections. We collected 121 blood samples from hunter-harvested or live-captured deer from two state-managed properties in northwest Florida, US; live captures were in support of a movement ecology study. Blood samples were tested for antibodies against titers to three EHDV serotypes (EHDV-1, EHDV-2, and EHDV-6), and multiplex quantitative reverse transcription PCR was used to identify the presence of EHDV or BTV viral RNA. Of these samples, 81% (98/121) tested seropositive for at least one of three serotypes of EHDV. Of those testing seropositive, 33% (40/121) contained antibodies for two serotypes, and 19% (24/121) contained antibodies for all three EHDV serotypes. Furthermore, results of generalized linear models indicated that the probability of infection by EHDV serotypes 1 and 6 increased with an animal's age. Our findings indicate that seroprevalence may be high for multiple serotypes in regions where these orbiviruses are endemic. These results could prove useful for managing disease risk in naïve deer populations.

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

Epizootic hemorrhagic disease viruses (EHDV) and bluetongue virus (BTV) are viral threats to white-tailed deer (WTD; *Odocoileus virginianus*) populations throughout their geographic distribution. These orbiviruses, which cause diseases commonly referred to as hemorrhagic disease (HD), are vectored by biting midges in the *Culicoides* genus. Although HD outbreaks, which can result in die-offs, have been documented in North America, little is known about population-level seroprevalence of this disease in regions where it is endemic, especially the southeastern US (Ruder et al. 2015). In fact, most available data regarding HD seroprevalence rates (Shope et al. 1960; Roughton 1975) are older and may not account for the changes in climate, vector range expansions, and discoveries of more recent novel serotypes. Hemorrhagic disease may not consistently cause severe die-offs in wild WTD populations in the southeastern US; however, it can manifest chronically, causing a range of other individual health issues, including hoof deformation, loss of appetite, oral lesions, and general lameness (Prestwood et al. 1974; Nol et al. 2010) that may significantly affect herd health. White-tailed deer populations in the more northern portions of North America may be at an increasingly greater risk of contracting HD as a result of global climate change–induced *Culicoides* range expansion, along with extended HD transmission seasons between *Culicoides* and WTD (Vigil et al. 2018; Allen et al. 2019). As such, a thorough understanding of HD exposure could aid those seeking to prevent and manage HD and similarly transmitted diseases in these regions. Our objectives were to 1) determine the seroprevalence of HD in native WTD in the Florida Panhandle and 2) examine trends between EHDV seroprevalence and age.

Whole blood samples were collected in 2017, 2018, and 2019 from December through March, from hunter-harvested and

live-sampled WTD on two state-managed properties located in Gadsden and Leon Counties, Florida (30°30′N, 84°30′W). Live captures were part of a separate movement ecology study targeting animals large enough to carry GPS collars safely. The properties on which we sampled included Joe Budd Wildlife Management Area (JBWMA), managed by the Florida Fish and Wildlife Conservation Commission, and Lake Talquin State Forest, which is managed by the Florida Forest Service. Hunting restrictions on JBWMA permit hunters to harvest only bucks with at least one antler with three or more points, and hunters are required to check their deer at a check station after harvest. Blood samples from these animals were collected by cardiac puncture with a 20-mL syringe with 18-gauge needle. Samples collected in December were pooled with the samples collected in the following 2 mo (January and February) of the hunting season for analysis. We supplemented this serologic dataset with WTD females and males of other age classes by darting and live-sampling from January through March on JBWMA and Lake Talquin State Forest before the onset of the HD season by the animal capture methodology detailed in Cauvin et al. (2020). Deer capture and handling protocols were developed by J.K.B. and approved by the University of Florida Institutional Animal Care and Use Committee (nos. 2015508838 and 201609412 to J.K.B.).

For both harvested and live-captured samples, blood samples were handled, sampled, stored, and processed with protocols detailed in Cauvin et al. (2020). Blood samples were tested for antibodies against titers to the three EHDV serotypes (EHDV-1, EHDV-2, and EHDV-6) observed in Florida by virus neutralization tests (Stallknecht et al. 1996) performed by the Texas A&M University Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA). Samples were considered seropositive for a specific serotype when titers were >20. Multiplex quantitative reverse transcription PCR (RT-PCR) was used to identify the presence of EHDV or BTV viral RNA (Wernike et al. 2015) on a portion of these samples by protocols detailed in Cauvin et al. (2020). A cycle threshold of ≤39 was considered positive.

We used Fisher's exact tests to determine differences ($P<0.05$) in seroprevalence between males and females (Fisher 1922; Kruskal and Wallis 1952; Dunn 1964) at each serotype for animals sampled in 2017 and 2018. Fisher's exact tests were also used to determine differences in seroprevalence between years for an overall pooled sample of males and females. Generalized linear models and the Hosmer-Lemeshow goodness of fit test ($g=9$) were used to investigate whether seroprevalence increased with age for each serotype, and the ggplot2 package (Wickham et al. 2018) in R 3.5.0 (R Core Team 2018) was used to plot the probability of exposure by age at capture in years. Exact binomial confidence intervals (CIs) for seroprevalence were calculated using the epitools package (Aragon et al. 2017) in R.

In total, we collected blood samples from 121 deer (Table 1). Except for the EHDV-6 serotype in 2018, Fisher's exact tests on individual years did not show significant differences in HD seroprevalence between male and female deer, allowing us to pool the samples for further analysis. Subsequent Fisher's tests between all years, with the pooled sample of both sexes, did not show significant differences in HD seroprevalence.

From all blood samples tested, 81% (98/121) tested seropositive for at least one serotype of EHDV in 2017, 2018, and 2019 combined. Of those testing seropositive, 33% (40/121) contained antibodies for two serotypes, and 19% (24/121) contained antibodies for all three serotypes. Serotype EHDV-2 was dominant between both sexes for each year, with a 71.1% overall seroprevalence for all years (Table 1). Generalized linear models showed that risks of antibody detection increased as an animal aged for EHDV-1 ($P=0.018$) and EHDV-2 ($P=0.009$). Risk of antibody development for EHDV-6 was not significantly influenced by age ($P=0.103$; Fig. 1). Hosmer-Lemeshow tests on the general-

TABLE 1. Epizootic hemorrhagic disease virus (EHDV) seroprevalence and 95% confidence interval (CI) of sample proportions for 121 wild white-tailed deer (*Odocoileus virginianus*) sampled by year on Joe Budd Wildlife Management Area and Lake Talquin State Forest in northwest Florida, USA.

				No. seropositive of EHDV serotypes (%; 95% CI)					
Year	Sex[a]	*n*	% Negative	EDHV-1	EDHV-2	EHDV-6	One serotype	Two serotypes	Three serotypes
2017	M	47	4 (8)	28 (59; 45–66)	37 (78; 65–88)	17 (36; 24–50)	13 (27; 16–41)	17 (36; 24–50)	14 (29; 18-44)
2017	F	18	5 (27)	8 (44; 24–66)	13 (72; 49–87)	6 (33; 16–56)	3 (16; 5–39)	6 (33; 16–56)	4 (22; 9-45)
2017	M, F	65	9 (13)	36 (55; 43–66)	50 (76; 65–85)	23 (34; 24–47)	16 (24; 15–36)	23 (35; 24–47)	18 (27; 18-39)
2018	M	19	3 (15)	9 (47; 27–68)	12 (63; 41–80)	12 (63; 41–80)	3 (15; 5.5–37)	7 (36; 19–59)	4 (21; 8-43)
2018	F	11	4 (36)	3 (27; 9–56)	6 (54; 28–78)	2 (18; 5–47)	4 (36; 15–64)	2 (18; 5–47)	1 (9; 1-37)
2018	M, F	30	7 (23)	12 (40; 24–57)	18 (60; 42–75)	14 (46; 30–63)	7 (23; 11–40)	9 (30; 16–47)	5 (16; 7-33)
2019	M	26	7 (26)	2 (7; 2–24)	18 (69; 50–83)	9 (34; 19–53)	10 (38; 22–57)	8 (30; 16–50)	1 (3; 0-18)
All	M	92	14 (15)	39 (42; 32–52)	67 (72; 63–80)	38 (41; 31–51)	26 (28; 20–38)	32 (34; 25–45)	19 (20; 13-30)
All	F	29	9 (31)	11 (37; 22–56)	19 (65; 47–80)	18 (62; 44–77)	20 (69; 50–82)	8 (27; 14–45)	5 (17; 7-34)
All	M, F	121	23 (19)	50 (41; 33–50)	86 (71; 62–78)	56 (46; 37–55)	46 (38; 29–46)	40 (33; 25–41)	24 (19; 13-27)

[a] M = male; F = female.

FIGURE 1. Generalized linear model of the relationship between epizootic hemorrhagic disease virus (EHDV) seroprevalence and age at capture for a wild white-tailed deer (*Odocoileus virginianus*) population in northwest Florida, USA, sampled in 2017–19. Gray shading represents the 95% confidence interval around estimates.

ized linear models showed EHDV-1 and EHDV-2 status were well predicted by age (P=0.454 and P=0.828, respectively), and EHDV-2 status was not predicted well by age (P=0.013). These results were consistent when the number of groups (g) was set to six to eight following suggestions detailed by Parzen and Lipsitz (1999).

For EHDV, 10% (6/56; 95% CI: 5–21%) of samples tested positive on RT-PCR in 2017, 14% (2/14; CI: 4%, 39%) in 2018, and 29% (5/17; CI: 13%, 53%) in 2019. Across the 3 yr, 92% (12/13; CI: 66%, 98%) of samples that tested positive on RT-PCR were seropositive for at least one serotype of EHDV. For BTV, about 2% (1/62; CI: 0%, 8%) of samples tested positive in 2017, 0% (0/16) in 2018, and about 5% (1/21; CI: 0%, 22%) in 2019.

Blood samples used in this study were collected opportunistically, because several samples were collected as part of a larger deer movement ecology study, and the rest were from hunter-harvest check stations. Despite this sampling, our data showed that EHDV exposure was high in the wild WTD populations of northwest Florida between 2017 and 2019, and we detected three endemic serotypes. Most animals sampled had antibodies to more than one serotype of

EHDV, which indicates that animals were being exposed to multiple serotypes of EHDV throughout their lifetime. This was supported by our observation that the probability of infection by EHDV-1 and EHDV-6 increases with an animal's age in years (Fig. 1). This trend of age-related co-infection could be of concern to herd managers because repeated exposure to novel serotypes of EHDV during an individual's lifetime could have negative effects on the animal's growth and fecundity. Additionally, the proportion of samples that tested positively for viral nucleic acid indicated a high overall risk of EHDV circulation among WTD populations in this region. A small proportion of samples also tested positive for BTV, suggesting a possible, albeit much lower, risk of BTV in this population. Our findings on the relationships between sex, age, and serotype exposure could be useful for WTD managers in the region seeking to understand HD outbreaks and its effect on wild populations.

Although investigations into the continuing range expansion of EHDV and BTV outbreaks in the northeast and midwest US have been made (Stallknecht et al. 2015), very few studies have conducted serologic surveys in the southeastern US, where HD is prevalent across deer populations. Additionally, our results provide insights into local exposure patterns within a herd over 3 yr. As a result, our seroprevalence results are novel and could serve as the foundation for future inquiries into how herd morbidity and mortality rates experienced in wild deer populations are affected by HD.

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