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Antibodies to Epizootic Hemorrhagic Disease Virus (EHDV) in Farmed and Wild Florida White-tailed Deer (*Odocoileus virginianus*)

Allison Cauvin,[1,5] Emily T. N. Dinh,[2,3,5] Jeremy P. Orange,[2,3] Rebecca M. Shuman,[4] Jason K. Blackburn,[2,3] and Samantha M. Wisely[1,6] [1]Department of Wildlife Ecology and Conservation, 110 Newins-Ziegler Hall, University of Florida, Gainesville, Florida 32611, USA; [2]Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, 3141 Turlington Hall, University of Florida, Gainesville, Florida 32611, USA; [3]Emerging Pathogens Institute, 2055 Mowry Road, University of Florida, Gainesville, Florida 32611, USA; [4]Florida Fish and Wildlife Conservation Commission, 1105 SW Williston Road, Gainesville, Florida 32601, USA; [5]These authors contributed equally to this work; [6]Corresponding author (email: wisely@ufl.edu)

ABSTRACT: The transmission of multiple serotypes of epizootic hemorrhagic disease virus (EHDV) between farmed and free-ranging wildlife is of interest to livestock industries and natural resource agencies. We compared the seroprevalence of EHDV-1, -2, and -6 in wild and farmed white-tailed deer (*Odocoileus virginianus*) herds in Florida, US. We compared serological prevalence, circulating serotypes, antibody titers, and viremia with the use of 171 whole-blood samples from 150 unvaccinated white-tailed deer from farm pens, a farm preserve, and wild deer on adjacent public lands between March 2016 and May 2017. Despite aggressive chemical vector control on the farm, we found higher seroprevalence and titers against the predominant EHDV serotype in farmed deer (in pens and the preserve) than in wild deer. The higher exposure to EHDV of farmed vs. wild deer may have been because of the higher densities of farmed vs. wild deer, the presence of exotic amplifying hosts such as elk (*Cervus canadensis*) in the preserve, or genetic factors that predisposed farmed deer to disease.

Key words: Biosecurity, Cervidae, deer farming, hemorrhagic disease, orbivirus.

Deer farming is a rapidly expanding rural industry in the US (Anderson et al. 2017). Farmed cervids are susceptible to a variety of infectious diseases, and the transmission of pathogens between both farmed and free-ranging wildlife is of concern to the captive cervid industry and natural resource management agencies. The most severe disease in captive deer is caused by epizootic hemorrhagic disease virus (EHDV), which is a double-stranded RNA virus in the *Orbiviridae* genus (Savini et al. 2011). The primary vector of EHDV is biting midges (*Culicoides* spp., Diptera: Ceratopogonidae) and EHDV is transmitted from late summer into fall during peak vector activity (Nettles et al. 1992). Three serotypes of EHDV are endemic to the US: EHDV-1, -2, and -6 (Ruder et al. 2017).

Comparing seroprevalence in wild and farmed white-tailed deer (*Odocoileus virginianus*) herds is an essential first step toward understanding the epidemiology of the virus and the risk of epizootic events. Our objectives were to determine whether farmed and native white-tailed deer from nearby (within about 4 km) state-managed properties exhibited differences in antibody prevalence, circulating serotypes, antibody titers, or viremia during 2016 and 2017 in the Florida panhandle. We hypothesized that higher population densities and intensive animal husbandry practices on the farm property would result in higher virus exposure and antibody titers than in wild deer, although chemical control of vectors within the farm but not on the publicly managed lands could negate or reverse this hypothesized epidemiological pattern.

We conducted our study within an approximately 200-ha privately owned, high-fenced deer farm in Gadsden County, Florida, US (Fig. 1). The property area included 10 high-fenced breeding pens that occupied about 8.5 ha and contained about 100 white-tailed deer (12 animals/ha) in each. Immediately surrounding the pens were 200 ha of Gulf Coast forest (the preserve) in which 130–150 free-ranging captive white-tailed deer (0.74 animals/ha) were managed with food plots and 12 supplemental protein feeders regularly filled by farm staff. In addition to white-tailed deer, the preserve had about 150 nonnative rumi-

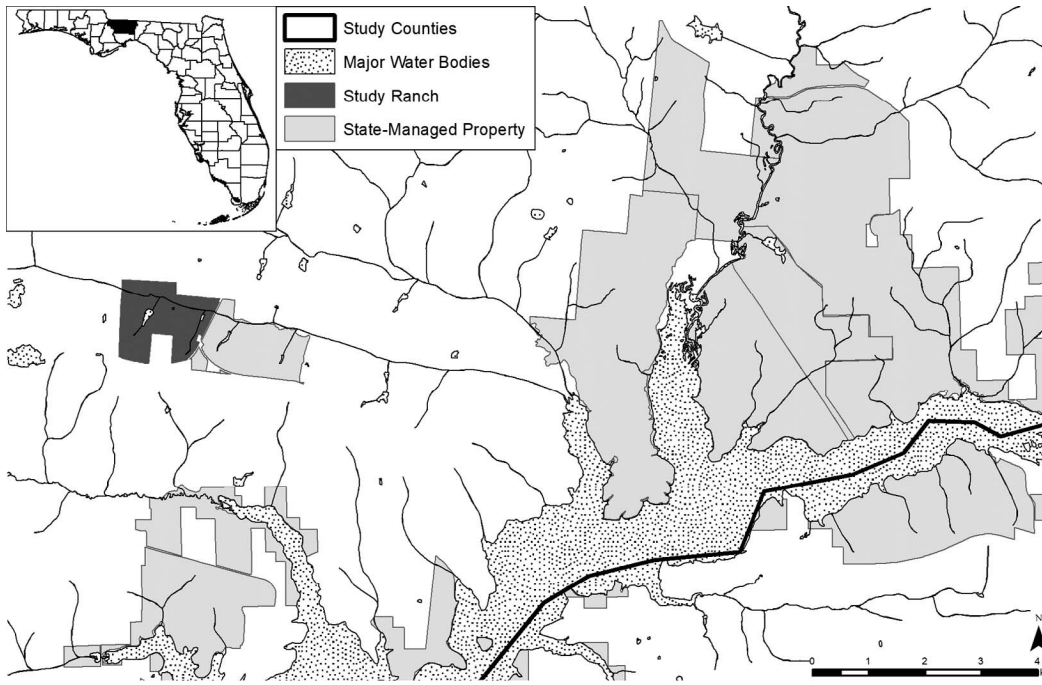

FIGURE 1. Relative location of a deer farm and nearby state-managed properties where farmed and wild white-tailed deer (*Odocoileus virginianus*) were studied in Gadsden and Leon counties, Florida, USA.

nants of 13 species (Bovidae and Cervidae; McGregor et al. 2018).

The farm manager used chemical vector control to reduce the incidence of EHDV transmission. Deer pens were treated with a misted solution of 75% Tengard SFR One Shot permethrin (UPI, King of Prussia, Pennsylvania, USA) and 25% Exponent (MGK, Minneapolis, Minnesota, USA) insecticide synergist solution. Permethrin application is the most utilized tactic for managing *Culicoides* midges (Pfannenstiel et al. 2015). This solution was applied in a twice-a-day, 2-wk-on, 1-wk-off schedule during the summer months (Cauvin et al. 2019). The adjacent farm preserve was occasionally misted with the same solution via all-terrain vehicle during summer.

We studied wild deer on Florida Fish and Wildlife Conservation Commission and Florida Forest Service properties within Gadsden and Leon counties, about 4 km from the study farm (Fig. 1). We estimated white-tailed deer at a density of 0.08 animals/ha. The management objectives of these public lands were multiple use: human recreation, timber harvest, and environmental needs.

We collected whole-blood samples (n=171) from 150 unvaccinated white-tailed deer between March and June in 2016 (n=80) and 2017 (n=91). Samples were collected from three groups of deer: 53% (91/150) penned deer, 16% (27/150) deer in the preserve, and 31% (53/150) wild deer. Only 21 individuals were bled twice (n=42 samples) in both 2016 and 2017. The remaining (n=129) samples were single samples from unique deer that were not resampled. Data from 2016 and 2017 were analyzed separately to maintain statistical independence of data.

The owner collected whole blood from penned deer during routine handling with the use of jugular venipuncture with 18–20-gauge needles. We immediately transferred the blood into BD Vacutainer serum separator tubes coated with clotting agent (Fisher

Scientific, Hampton, New Hampshire, USA) and subsequently into 1-mL ethylenediaminetetraacetic acid (EDTA)–coated tubes. Blood was centrifuged and serum was separated on site and stored at –20 C within 48 h of collection. Whole blood collected into EDTA tubes for reverse-transcription PCR was stored at 4 C for <48 h prior to storage at –80 C.

We chemically immobilized preserve and wild deer with projectile darts loaded with 1.5–2.0 mL premixed butorphanol tartrate–azaperone tartrate–medetomidine HCl (BAM; Wildlife Pharmaceuticals, Windsor, Colorado, USA). We reversed the BAM with 0.5 mL Naltrexone HCl and twice the dosage of BAM with atipamezole HCL (Wildlife Pharmaceuticals) delivered via intramuscular injection in the shoulder or hindquarter. We collected and handled samples identically as they were for penned deer.

We extracted RNA from whole blood in EDTA using a standard magnetic bead protocol (Kingfisher Duo Prime; Thermo Fisher Scientific, Waltham, Massachusetts, USA). Multiplex quantitative reverse transcription–PCR (qRT-PCR) was used to determine the presence of EHDV or BTV RNA (Wernike et al. 2015). A cycle threshold value ≥40 was considered negative and a cycle threshold ≤39 was considered positive.

Antibody titers against EHDV-1, -2, and -6 were determined with the use of a virus neutralization test (Stalknecht et al. 1996) at the Texas A&M University Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA). Antibody titer was expressed as the reciprocal of the highest serum dilution at which cytopathic effects were inhibited. Negative samples (nonreactors) were designated as zeroes and included in our comparative titer analyses. Animals were considered seronegative when titers were <20.

All statistical analyses were completed in R version 3.4.0 (R Core Team 2016) and figures were generated with the 'ggplot2' package in R (Wickham 2016). We used the Fisher's exact and Kruskal–Wallis tests with Dunn's post hoc analysis to determine differences in seroprevalence and titer levels, respectively (Fisher 1922; Kruskal and Wallis 1952; Dunn 1964).

In 2016, EHDV-1 was the predominant serotype, and during this period, significantly more penned (P=<0.001) and preserve (P=<0.001) deer were seropositive for EHDV-1 than wild deer. For EHDV-2, preserve deer had significantly higher prevalence than either penned (P=0.002) or wild (P=0.004) deer, and preserve deer had a higher prevalence than wild deer (P=0.021) for EHDV-6 (Table 1). During 2017, EHDV-2 was the predominant serotype, and both preserve (P=0.047) and penned (P=0.006) deer had significantly higher seroprevalence than wild deer for EHDV-2. In both years, the most prevalent EHDV serotype was the same in farmed deer and wild deer, and farmed deer had significantly higher exposure levels to whichever EHDV serotype was predominant (Table 1).

Titers to EHDV serotypes followed the same overall trend as seroprevalence. Farmed deer had significantly higher (P<0.001) titers to EHDV-1 than did wild deer in 2016. In addition, penned and preserve WTD had significantly different (P=0.026) titers to EHDV-1 in 2016. However, only penned and wild deer had significantly different (P=0.034) titers to EHDV-2 in 2017 (Fig. 2).

No deer from any herd had detectable EHDV nucleic acids in 2016. In 2017, 3% (1/39) of penned deer, 0% (0/12) of deer from the preserve, and 5% (2/40) of wild deer were positive for EHDV nucleic acids in blood samples. No animal was positive for BTV. The prevalence of viral nucleic acid was not significantly different for farmed or wild deer (P=1.00 for penned vs. wild; P=1.00 for preserve vs. wild; P=1.00 for penned vs. preserve).

We demonstrated that the predominant EHDV serotype was synchronous among herds of wild and farmed (penned and preserve) deer in close proximity. In 2016, EHDV-1 was the predominant serotype in both farmed and wild deer, but the predominant serotype switched to EHDV-2 in both farmed and wild deer in 2017. Farmed deer, however, had both higher seroprevalence and

TABLE 1. The number of white-tailed deer (*Odocoileus virginianus*) seropositive for epizootic hemorrhagic disease virus, the total number of deer surveyed, seroprevalence, and the 95% confidence interval (CI) of seroprevalence of epizootic hemorrhagic disease virus by year and serotype in white-tailed deer from Florida, USA, 2016 and 2017. Additive percentages are >100% because of detection of multiple serotypes per individual. The *P* values indicate the result of Fisher's exact test; significant differences are indicated in bold.

		2016				2017			
Source	EHDV[a]	% Positive (*n* positive/ *n* tested)	95% CI	*P*	Significant differences[b]	% Positive (*n* positive/ *n* tested)	95% CI	*P*	Significant differences[b]
Penned	1	92 (48/52)	81–98	**<0.001**	**A**	49 (19/39)	32–65	0.173	A
				0.568	C			0.510	C
	2	50 (26/52)	36–64	0.543	A	95 (37/39)	83–99	**0.006**	**A**
				0.002	**C**			1.000	C
	6	48 (25/52)	34–62	0.127	A	21 (8/39)	9–37	1.000	A
				0.141	C			0.442	C
Preserve	1	100 (15/15)	78–100	**<0.001**	**B**	33 (4/12)	10–65	1.000	B
				0.568	C			0.510	C
	2	93 (14/15)	64–100	**0.004**	**B**	100 (12/12)	73–100	**0.047**	**B**
				0.002	**C**			1.000	C
	6	73 (11/15)	45–92	**0.021**	**B**	33 (4/12)	10–66	0.466	B
				0.141	C			0.442	C
Wild	1	39 (5/13)	14–68	**<0.001**	**A**	33 (13/40)	19–49	**0.006**	**A**
				<0.001	**B**			0.510	B
	2	39 (5/13)	14–68	0.543	A	70 (28/40)	53–83	**0.006**	**A**
				0.004	**B**			**0.047**	**B**
	6	23 (3/13)	5–54	0.127	A	23 (9/40)	11–39	1.000	A
				0.021	**B**			0.466	B

[a] EHDV = epizootic hemorrhagic disease virus serotype.

[b] A indicates the comparison between penned vs. wild; B indicates the comparison between preserve vs. wild; C for the comparison between penned vs. preserve.

titers to the predominant serotype of that year. Within the farm, there was no difference in titers between penned and preserve deer except for EHDV-2 in 2016; penned deer had lower antibody response to exposure to EHDV-2 than preserve deer.

The higher prevalence of antibodies to EHDV in farmed deer may be the result of several factors. Farmed deer were at higher densities than wild deer, which is conducive to viral transmission. The presence of other amplifying hosts for EHDV such as elk (*Cervus canadensis*; Hoff and Trainer 1973) on the preserve may have contributed to an increase in pathogen exposure of farmed deer. Although the general attributes of the Gulf Coast forest ecosystem were similar throughout the study area, we cannot rule out differences in site-specific habitat that could have contributed to different densities of *Culicoides* vectors.

Despite the use of chemical vector control, we found higher exposure to EHDV in farmed white-tailed deer than in a wild herd adjacent to the farm in two successive years. This observation suggests that current vector control strategies are not limiting EHDV exposure in farmed white-tailed deer, demonstrating the need for improved integrated pest management strategies.

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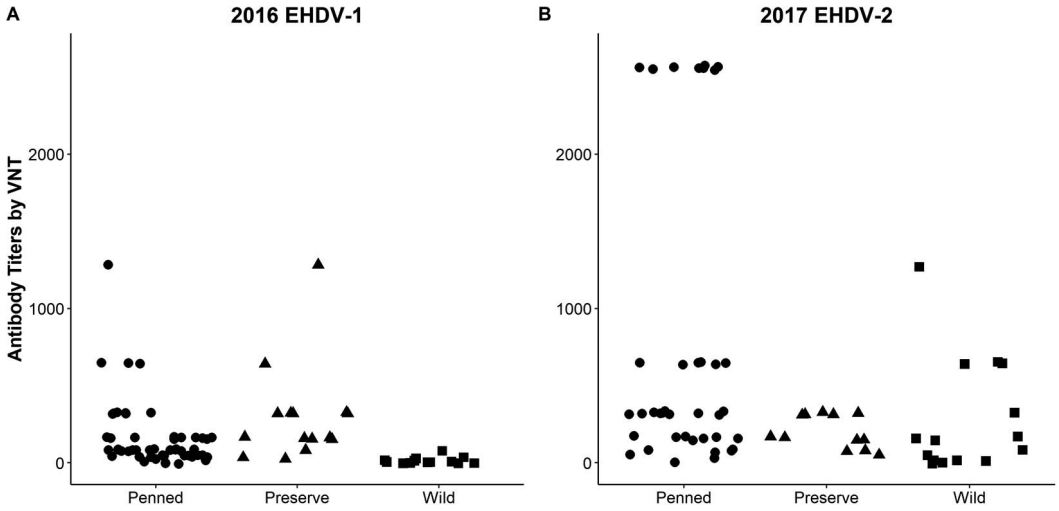

FIGURE 2. Antibody titers across cohorts of white-tailed deer (*Odocoileus virginianus*) in Gadsden and Leon counties, Florida, USA. Penned and preserve deer had significantly higher ($P<0.001$) titers to epizootic hemorrhagic disease virus (EHDV)-1 in 2016 than did wild deer, and penned and preserve WTD had significantly different ($P=0.026$) titers to EHDV-1 in 2016. In 2017 only penned and wild deer had significantly different ($P=0.034$) titers to EHDV-2.

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