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EVIDENCE OF EPIZOOTIC HEMORRHAGIC DISEASE VIRUS AND BLUETONGUE VIRUS EXPOSURE IN NONNATIVE RUMINANT SPECIES IN NORTHERN FLORIDA

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Abstract: Epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) are vector-borne viruses of ruminants nearly worldwide. They can affect white-tailed deer (WTD; *Odocoileus virginianus*), the ranching industry, and nonindigenous hoof stock species managed for conservation. One potential risk factor for ranched WTD is commingling with nonindigenous species on high-fenced properties. Nonindigenous species provide novel viewing and hunting opportunities; however, their presence may create disease hazards. Furthermore, animals within conservation properties may be at a risk from commingling exotics and adjacent wild WTD. Currently, knowledge about EHDV and BTV seroprevalence and transmission is limited in nonindigenous populations in the southeastern United States. The authors conducted a serological survey of 10 Bovidae and 5 Cervidae species residing within two properties in northern Florida. The first site was a conservation property breeding threatened nonindigenous species for conservation. The second property was a private high-fenced game preserve managing WTD and nonindigenous species for breeding, sale, and harvest. Blood samples were tested for titers to three EHDV serotypes (1, 2, and 6) and active circulating viral EHDV and BTV. The private ranch had evidence of EHDV or BTV in one of three (33.3%) Bovidae species and four of five (80%) Cervidae species sampled. At the conservation property, evidence of EHDV infection was found in four of seven (57.1%) Bovidae and one of one (100%) Cervidae species sampled. The presence of antibodies in many nonindigenous species sampled might indicate these species are potential viral hosts and may be a risk to ranched WTD and other species within the same property. Nonindigenous species within the private ranch and conservation properties are at risk of contracting EHDV and BTV, and herd managers should reduce vector–host interactions and consider increased biosecurity measures when translocating animals.

INTRODUCTION

Epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) pose threats to captive, domestic, and wild ruminants throughout the world.[13,27,32] Currently 27 BTV[4] and 7–10 EHDV[1,20] serotypes are recognized worldwide. EHDV and BTV are vectored by the *Culicoides* family of biting midges, with more than 1,400 identified species found on almost every continent in the world.[19] Vaccines (autogenous vaccines) are available for some BTV[12] and EHDV serotypes; however, their efficacy may be low[38] because vaccines may be specific to only certain serotypes, and vaccination may be impossible in most wild, semiwild, or free-ranging herds.

In North America, EHDV and BTV can cause severe clinical signs (e.g. hemorrhaging, edema, hoof-sloughing, oral lesions, and death) in white-tailed deer (WTD; *Odocoileus virginianus*) populations.[28,34] WTD ranching industry herds may be at greater risk than wild populations because animals within high-fenced properties are commonly stocked at higher density than wild WTD populations, which may facilitate increased exposure. For example, within northwestern Florida, EHDV seroprevalence was higher in ranched WTD than in an adjacent wild WTD population.[6] Transmission dynamics within high-fenced ranches may be further amplified by the presence and close contact of nonindigenous and domesticated species that could act as reservoir hosts for orbiviruses.[21] Like high-fenced game ranches, animals within conservation properties are often kept at unnaturally high animal densities, which may further facilitate disease spread.

Throughout North America, many private farms raise nonindigenous bovid and cervid species in addition to WTD on high-fenced properties.[10,14,24] Nonindigenous animal numbers on farms can range from a few individuals that

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provide novelty to many individuals of variable species that may be a significant proportion of the animals within the property. Although nonindigenous species provide additional novel hunting or viewing experiences, their presence may have negative effects on WTD by becoming a source of interspecific competition,[11] novel disease introductions, or hybridization[5] or by potentially acting as diseases reservoirs.

Both EHDV and BTV are significant viral threats to nonindigenous ruminant species in North America held by conservation and zoological organizations. Nonindigenous species are not only vulnerable to native pathogens, they are also potential reservoirs for native pathogens or sources of novel pathogens that could be transmitted to wild and domesticated ruminant populations.[9,16,36]

Conservation organizations need to be vigilant of disease introduction and spread during conservation and translocation efforts. A thorough disease risk analysis is highly recommended before any reintroduction or translocation effort[3,15] on the basis of a thorough understanding of EHDV and BTV epidemiology on conservation properties, including those species that may act as disease reservoirs.

Although experimental EHDV and BTV infections have been demonstrated in some nonindigenous species, very little is known about EHDV and BTV epidemiology in most nonindigenous species that are exposed to endemic pathogens on private ranches and conservation properties in the southeastern United States. Furthermore, documentation of EHDV or BTV exposure in ruminant species inhabiting their native geographic ranges could be misleading because individuals may encounter different serotypes in geographical regions outside of their native range. In central Texas, titers to EHDV and BTV were found in 16% and 4% of axis deer (*Axis axis*) and 64% and 57% of fallow deer (*Dama dama*) sampled.[22] In the southeastern United States, four of five (80%) fallow deer had titers to BTV and none had titers to EHDV.[35] During a nationwide survey, BTV was found by polymerase chain reaction (PCR) in gerenuk (*Litocranius walleri*) living in north America.[23] Elk (*Cervus canadensis*) have had a wide range of EHDV and BTV serological results. In Kentucky, sampled elk prevalence was 0% for BTV and 3.2% for EHDV,[7] whereas in Arkansas, titers to BTV and EHDV were detected in 12.9% and 20% of the animals sampled.[7] In Nebraska, 11% and 12% of hunter-harvested elk in Nebraska had titers to BTV and EHDV, respectively.[8]

The geographic distribution of competent vectors varies significantly, which might impact viral transmission to nonindigenous hosts. For example, *Culicoides sonorensis*, a competent EHDV vector in much of the United States[26] is rare in some regions of the southeastern United States,[30,31] and *Culicoides stellifer* and *Culicoides venustus* have been identified as the competent EHDV vectors of concern in Florida and Alabama.[17] Furthermore, host use preferences may vary between *Culicoides* species. In northwest Florida, *C. stellifer* preferred elk and fallow deer while avoiding Bovidae species, and host use preference varied between the *Culicoides* species sampled and from year to year.[18] The dynamic nature of vector ecology and vector–host interactions in this disease system further complicates the epidemiology of these orbiviruses for nonindigenous species. The objective of this study was to better understand EHDV and BTV seroprevalence in nonindigenous species in the southeastern United States and identify potential disease risk factors to the private WTD deer industry and to nonindigenous ruminant conservation efforts.

MATERIALS AND METHODS

Blood samples were collected from two sites. One site was an ~200-ha privately owned ranch in Gadsden County, FL. The ranch was separated into a 20-ha ranch breeding center with ~100 WTD enrolled in a ranch breeding program and a 180-ha big-game preserve stocked with ~130–150 WTD, 30–40 blackbuck antelope (*Antilope cervicapra*), 6–8 nilgai (*Boselaphus tragocamelus*), 6–8 scimitar-horned oryx (*Oryx dammah*), 7–9 gemsbok (*Oryx gazella*), 40 axis deer, 19–22 *Cervus* spp. (Rocky Mountain elk [*C. canadensis*]/Sika [*Cervus nippon*]/sika–elk hybrids), 3 goats (*Capra hircus*), 3 bighorn sheep (*Ovis aries*), 12–24 fallow deer, and 7–9 Père David's deer (*Elaphurus davidianus*).[18] The entire 200-ha property was enclosed with a 3-m-tall fence. Within the preserve portion of the ranch, sampling was done in spring (April and May) at the beginning of the EHDV and BTV transmission season and in the fall (September–November) toward the end of the transmission season. Blood samples were also collected by cardiac puncture from ranched animals that died during the two sampling periods.

Animals on the private ranch were sedated with 1.0–3.0 ml butorphanol tartrate–azaperone tartrate–medetomidine HCl (Wildlife Pharmaceuticals, Windsor, CO 80550, USA) following manufacture-suggested dosage guidelines. Gel-collared or double-barbed transmitter darts

(Pneu-Dart Inc., Williamsport, PA 17701, USA) were used, depending on the species darted and the sampling strategy. Some animals were captured in more than one season or year. Blood was collected from animals by jugular venipuncture with a 20-ml syringe and 18-ga needle. Blood was transferred from syringes into 6-ml serum separator tubes (Thermo Fisher Scientific, Waltham, MA 02451, USA) and 1–3-ml EDTA tubes (Thermo Fisher Scientific) immediately after collection. Whole blood samples were collected from three Bovidae species (blackbuck antelope [$n = 5$], nilgai [$n = 2$], and scimitar oryx [$n = 2$]) and five Cervidae species (WTD [$n = 47$], Père David's deer [$n = 10$], elk [$n = 7$], axis deer [$n = 6$], and fallow deer [$n = 4$]) from 2015 to 2018. For this study, elk were considered a nonindigenous species because they are not native to the Gulf Coast Plains in the southeastern United States. Some animals were sampled during multiple seasons.

The second site was a large conservation property in Nassau County, FL, that manages numerous threatened or endangered African ungulate and carnivore species. Currently, the property houses about 45 species with a total animal enclosure area of ~283 ha. Enclosure sizes range from 0.4 ha up to about 6 ha, depending on species and herd sizes. Animals from this property are translocated to other conservation properties throughout the United States and are used for occasional reintroduction efforts in Africa (e.g. South Africa, Kenya, and Zimbabwe). Blood samples were collected year round during routine animal handling activities (e.g. movements, health checks). Blood was collected by jugular venipuncture with a 20-ga needle and drawn directly into serum separator tubes (Fisher Scientific, Hampton, NH, USA). Samples were collected from seven Bovidae species (dama gazelle [*Nanger dama*; $n = 8$], gerenuk [n = 3], lesser kudu [*Tragelaphus imberbis*; $n = 5$], Nile lechwe [*Kobus megaceros*; $n = 2$], roan antelope [*Hippotragus equinus*; $n = 7$], slender-horned gazelle [*Gazella leptoceros*; $n = 3$], and bongo antelope [*Tragelaphus eurycerus*; $n = 4$]) and one Cervidae species (Père David's deer [$n = 2$]) from 2016 to 2018.

Blood samples were handled, stored, and processed following protocols detailed by Cauvin et al.[6] Disease exposure testing was done by virus neutralization assays at the Texas Veterinary and Medical Diagnostic Laboratory, College Station, TX. Samples were considered negative for a specific EHDV serotype (1, 2, or 6) when titers were <20. Multiplex quantitative reverse transcription PCR (qRT-PCR) was used to identify the presence of EHDV or BTV viral RNA[37] from most samples collected on the private ranch by protocols detailed in Cauvin et al.[6] A threshold cycle (C_T) of ≤ 39 was considered positive. The presence of BTV or EHDV viral RNA indicates circulating virus, whereas the presence of antibodies indicates past or current exposure, or both.

Samples were grouped into early season (February through May) and late season (August through November) time periods, and any samples collected outside of these time windows were censored. The early season corresponds to the beginning of the EHDV and BTV transmission season, and the late season corresponds to the approximate end of the transmission season in the United States.[27] Winter freezes in late November and early December typically result in reduced midge activity and the end of the EHDV and BTV transmission season; however, transmission can continue year round in the subtropical southeastern United States.[29] Prevalence with 95% confidence intervals (CI) was estimated for any species with 10 or more individuals sampled. When an individual was sampled multiple times during the study, only the serological results from its first capture event were included when calculating CI for the proportion positive. CI for the population proportion were estimated by the *epitools* package[2] in R version 3.6.3.[25]

RESULTS

Titers to EHDV were identified for at least one serotype in five of 10 (50%) Bovidae species and four of five (80%) Cervidae species sampled (Table 1). Viral RNA of BTV or EHDV was found in Rocky Mountain elk and white-tailed deer by PCR.

Within the private ranch, EHDV titers were found in one of three (33.3%) Bovidae species and four of five (80%) Cervidae species sampled. At the conservation property, titers to EHDV were found in four of seven (57.1%) Bovidae and one of one (100%) Cervidae species sampled. EHDV seroprevalence was high in ranched WTD on the private ranch, with up to 100% prevalence during a given season.

Prevalence with CI was estimated for two of the species sampled. Eight of 12 Père David's deer sampled (66.7%; CI: 39.1%–86.2%) had titers to EHDV-1, 11 (91.7%; CI: 64.6%–98.5%) had titers to EHDV-2, and one (8.3%; CI: 1.5%–35.4%) had titers to EHDV-6. No evidence of EHDV or BTV viral RNA was found by PCR in any Père David's deer sampled. Of the 47 WTD sampled, 25

Table 1. Epizootic hemorrhagic disease virus (EHDV-1, -2, or -6) seroprevalence and EHDV/bluetongue virus (BTV) viral RNA presence, for 10 Bovidae species and five Cervidae species from 2015 to 2018 on a private ranch and a conservation property in northern Florida.[a]

Year	Season	Species	Scientific name	Sampling location	*n*	EHDV-1 pos.	EHDV-2 pos.	EHDV-6 pos.	EHDV RT-PCR pos.	BTV RT-PCR pos.
Bovidae										
2015	Early	Blackbuck antelope	*Antilope cervicapra*	Private ranch	2	0 (0%)	0 (0%)	1 (50.0%)	NA	NA
2015	Late	Blackbuck antelope	*Antilope cervicapra*	Private ranch	1	0 (0%)	0 (0%)	0 (0%)	NA	NA
2017	Late	Blackbuck antelope	*Antilope cervicapra*	Private ranch	3[b]	NA	NA	NA	0/3 (0%)	0/3 (0%)
2017	Late	Bongo antelope	*Tragelaphus eurycerus*	Conservation property	4	0 (0%)	1 (25.0%)	0 (0%)	NA	NA
2017	Late	Dama gazelle	*Nanger dama*	Conservation property	8	1 (12.5%)	2 (25.0%)	3 (37.5%)	NA	NA
2016	Late	Gerenuk	*Litocranius walleri*	Conservation property	2	0 (0%)	0 (0%)	0 (0%)	NA	NA
2017	Late	Gerenuk	*Litocranius walleri*	Conservation property	1	0 (0%)	0 (0%)	0 (0%)	NA	NA
2018	Early	Lesser kudu	*Tragelaphus imberbis*	Conservation property	4	1 (25%)	2 (50%)	1 (25%)	NA	NA
2017	Late	Lesser kudu	*Tragelaphus imberbis*	Conservation property	1	0 (0%)	0 (0%)	0 (0%)	NA	NA
2018	Early	Nile lechwe	*Kobus megaceros*	Conservation property	2	0 (0%)	0 (0%)	0 (0%)	NA	NA
2016	Late	Nilgai	*Boselaphus tragocamelus*	Private ranch	1[b]	NA	NA	NA	0/1 (0%)	0/1 (0%)
2017	Late	Nilgai	*Boselaphus tragocamelus*	Private ranch	1	0 (0%)	0 (0%)	0 (0%)	0/1 (0%)	0/1 (0%)
2018	Early	Roan antelope	*Hippotragus equinus*	Conservation property	3	0 (0%)	1 (33.3%)	0 (0%)	NA	NA
2017	Late	Roan antelope	*Hippotragus equinus*	Conservation property	4	1 (25.0%)	1 (25.0%)	0 (0%)	NA	NA
2016	Late	Scimitar-horned oryx	*Oryx dammah*	Private ranch	1[b]	NA	NA	NA	0/1 (0%)	0/1 (0%)
2017	Late	Scimitar-horned oryx	*Oryx dammah*	Private ranch	1	0 (0%)	0 (0%)	0 (0%)	0/1 (0%)	0/1 (0%)
2016	Late	Slender-horned gazelle	*Gazella leptoceros*	Conservation property	1	0 (0%)	0 (0%)	0 (0%)	NA	NA
2017	Late	Slender-horned gazelle	*Gazella leptoceros*	Conservation property	2	0 (0%)	0 (0%)	0 (0%)	NA	NA
Cervidae										
2015	Early	Axis deer	*Axis axis*	Private ranch	2	0 (0%)	0 (0%)	0 (0%)	NA	0/2 (0%)
2017	Early	Axis deer	*Axis axis*	Private ranch	3	0 (0%)	0 (0%)	0 (0%)	0/3 (0%)	0/3 (0%)
2015	Late	Axis deer	*Axis axis*	Private ranch	2	0 (0%)	0 (0%)	0 (0%)	NA	0/2 (0%)
2017	Late	Axis deer	*Axis axis*	Private ranch	1	0 (0%)	0 (0%)	0 (0%)	0/1 (0%)	0/1 (0%)
2015	Early	Elk	*Cervus canadensis*	Private ranch	2	0 (0%)	1 (50.0%)	0 (0%)	NA	NA
2015	Late	Elk	*Cervus canadensis*	Private ranch	2	1 (50%)	2 (100%)	1 (50%)	NA	NA
2017	Late	Elk	*Cervus canadensis*	Private ranch	6	5 (83.3%)	6 (100%)	5 (83.3%)	1/6 (16.7%)	0/6 (0%)
2015	Early	Fallow deer	*Dama dama*	Private ranch	2	0 (0%)	0 (0%)	1 (50.0%)	NA	NA
2016	Early	Fallow deer	*Dama dama*	Private ranch	1	0 (0%)	1 (100%)	0 (0%)	0/1 (0%)	0/1 (0%)
2017	Early	Fallow deer	*Dama dama*	Private ranch	1	0 (0%)	1 (100%)	0 (0%)	0/1 (0%)	0/1 (0%)
2015	Late	Fallow deer	*Dama dama*	Private ranch	2	1 (50%)	1 (50%)	0 (0%)	NA	NA
2017	Late	Fallow deer	*Dama dama*	Private ranch	1[b]	NA	NA	NA	0/1 (0%)	0/1 (0%)
2015	Early	Père David's deer	*Elaphurus davidianus*	Private ranch	1	0 (0%)	1 (100%)	0 (0%)	NA	NA
2016	Early	Père David's deer	*Elaphurus davidianus*	Private ranch	1	1 (100%)	1 (100%)	0 (0%)	0/1 (0%)	0/1 (0%)
2017	Early	Père David's deer	*Elaphurus davidianus*	Private ranch	7	3 (42.9%)	6 (85.7%)	0 (0%)	0/7 (0%)	0/7 (0%)
2018	Early	Père David's deer	*Elaphurus davidianus*	Conservation property	1	1 (100%)	1 (100%)	0 (0%)	NA	NA

Table 1. Continued.

Year	Season	Species	Scientific name	Sampling location	*n*	EHDV-1 pos.	EHDV-2 pos.	EHDV-6 pos.	EHDV RT-PCR pos.	BTV RT-PCR pos.
2015	Late	Père David's deer	*Elaphurus davidianus*	Private ranch	1	0 (0%)	1 (100%)	0 (0%)	NA	NA
2016	Late	Père David's deer	*Elaphurus davidianus*	Private ranch	1	1 (100%)	1 (100%)	0 (0%)	0/1 (0%)	0/1 (0%)
2017	Late	Père David's deer	*Elaphurus davidianus*	Private ranch	2	2 (100%)	2 (100%)	1 (50.0%)	0/2 (0%)	0/2 (0%)
2017	Late	Père David's deer	*Elaphurus davidianus*	Conservation property	1	1 (100%)	1 (100%)	0 (0%)	NA	NA
2015	Early	White-tailed deer	*Odocoileus virginianus*	Private ranch	11	0 (0%)	10 (90.9%)	1 (9.1%)	3/11 (27.3%)	5/11 (45.5%)
2016	Early	White-tailed deer	*Odocoileus virginianus*	Private ranch	15	15 (100%)	14 (93.3%)	11 (73.3%)	0/15 (0%)	0/15 (0%)
2017	Early	White-tailed deer	*Odocoileus virginianus*	Private ranch	12	4 (33.3%)	12 (100%)	4 (33.3%)	0/12 (0%)	0/12 (0%)
2018	Early	White-tailed deer	*Odocoileus virginianus*	Private ranch	7	6 (85.7%)	7 (100%)	6 (85.7%)	NA	NA
2015	Late	White-tailed deer	*Odocoileus virginianus*	Private ranch	11	10 (90.9%)	10 (90.9%)	1 (9.1%)	3/11 (27.3%)	5/11 (45.5%)
2016	Late	White-tailed deer	*Odocoileus virginianus*	Private ranch	12	12 (100%)	12 (100%)	11 (91.7%)	0/12 (0%)	5/12 (41.7%)
2017	Late	White-tailed deer	*Odocoileus virginianus*	Private ranch	17	11 (64.7%)	16 (94.1%)	10 (58.8%)	6/17 (35.3%)	6/17 (35.3%)

[a] pos., positive; RT-PCR, reverse transcription polymerase chain reaction; NA, not applicable.
[b] Individuals were sampled postmortem.

(53.2%; CI: 39.2%–66.7%) had titers to EHDV-1, 44 (93.6%; CI: 82.8%–97.8%) had titers to EHDV-2, and 21 (44.7%; CI: 31.4%–58.8%) had titers to EHDV-6. Evidence of EHDV viral RNA was found in seven of 44 (16.0%; CI: 7.9%–29.4%) WTD, and BTV viral RNA was found in 10 of 44 (22.7%; CI: 12.8%–37.0%) WTD.

DISCUSSION

Antibodies to EHDV were observed in 60% of the species tested, and to our knowledge constitute some of the first known published cases of EHDV exposure to serotypes 1, 2, or 6 in these species in the southeastern United States. Sample sizes were too limited to rule out those species that may be resistant to infection or exposure to EHDV and BTV; however, it is possible that behavioral mechanisms or physiological differences may make these species less likely to encounter competent vectors in the habitats they occupy. Sample sizes were too small to allow conclusions about species variation; however, some of the species sampled (e.g. Père David's deer and elk) had similar EHDV seroprevalence as ranched WTD. Nonindigenous animals provide ranch owners with novel hunting and viewing experiences; however, ranchers should be aware that these species may be EHDV hosts and may constitute health risks to ranched WTD. Nonindigenous species that appear to be potential reservoirs could be contributing to the transmission cycle on the ranch, which decreases WTD health and reproductivity.

Within the conservation property, EHDV titers were observed in 57.1% of the Bovidae and 100% of the Cervidae species sampled. Movement of nonindigenous species with titers to EHDV from the conservation property to other conservation properties in different regions or back to areas of their native range as part of reintroduction efforts may pose a risk of introducing regionally novel EHDV or BTV serotypes to the translocation destination. Conversely, naïve animals imported to north Florida may be susceptible to infection and subsequent disease. Currently, no illness or death from EHDV or BTV have been no documented in nonindigenous species at the conservation property; however, clinical signs have been documented in nonindigenous species existing in North America.[33] To reduce potential EHDV spread and protect animal health, it may be important to screen animals extensively and universally before movement to determine their exposure status and limit movements and translocation efforts to times when EHDV and BTV

are not actively circulating. In the southeastern United States, where transmission has been documented year round[29] and where active circulating BTV and EHDV viral RNA were observed in ranched WTD during the early sampling season, the time window may be limited. Should vaccines for these viruses become available, vaccination of animals to be transported may be prudent. The results of our study highlight the need for more in-depth serological surveys and monitoring of nonindigenous ruminants on conservation and private properties throughout North America.

Ranched WTD had some of the highest rates of active EHDV or BTV infection or evidence of past infection. Postmortem examinations were conducted on many of the animals that died within the private ranch from 2015 to 2020, and EHDV and BTV were both associated with WTD mortalities. In multiple seasons, almost all WTD sampled on the private ranch had antibodies to one or more EHDV serotypes. Furthermore, circulating viral RNA was observed at high rates (>40%) in multiple seasons, suggesting ranched WTD are a risk factor for naïve nonindigenous species coexisting on these two properties. Within conservation and private properties with threatened nonindigenous ruminants, it may be best to reduce ranched WTD numbers, if they are present, to remove one potential source of viral infection. Similarly, understanding proximity of nonindigenous rearing operations and wild WTD herds is important for understanding exposure risk because wild WTD are hosts for EHDV and BTV and often have high EHDV infection rates.[6]

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