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Host associations of biting midges (Diptera: Ceratopogonidae: *Culicoides*) at deer farms in Florida, USA

Kristin E. Sloyer[1,*,], Carolina Acevedo[1], Samantha M. Wisely[2], Nathan D. Burkett-Cadena[1,]

[1]Florida Medical Entomology Laboratory, University of Florida IFAS, 200 9th St. SE, Vero Beach, FL, USA, [2]Department of Wildlife Ecology and Conservation, University of Florida, 110 Newins-Ziegler Hall, Gainesville, FL, USA *Corresponding author, mail: ksloyer@ufl.edu

Documenting the host use of vector species is important for understanding the transmission dynamics of vector-borne pathogens. Biting midges (Diptera: Ceratopogonidae: *Culicoides*) are vectors of epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) worldwide. However, relative to mosquitoes and many other vector groups, host associations of this group are poorly documented. In this study, we used PCR-based bloodmeal analysis to determine species-level host associations of 3,603 blood-engorged specimens belonging to 18 *Culicoides* species at 8 deer farms in Florida, USA. We used a binomial mixed model with a Bayesian framework to compare the effect of host composition on the feeding patterns of *Culicoides* spp. and employed the Morisita-Horn Index to investigate the similarity of host use between farms for *Culicoides stellifer* and *Culicoides insignis*. Results show that the estimated probability of *Culicoides* spp. feeding upon white-tailed deer depends on the availability of cattle or exotic game and demonstrates differences in host-feeding selection among species. *Culicoides insignis* had high host similarity across farms suggesting that its host-use patterns are somewhat conserved. *Culicoides stellifer* had lower host similarity across farms suggesting that it is a more opportunistic feeder. White-tailed deer are fed upon by many *Culicoides* species on deer farms in Florida, and while most *Culicoides* species feed on white-tailed deer, the ratio of white-tailed deer bloodmeals to other bloodmeals is likely influenced by host availability. *Culicoides* spp. taking a majority of their bloodmeals from farmed white-tailed deer should be assessed for their vector competence for EHDV and BTV.

Key words: Culicoides, biting midge, white-tailed deer, Epizootic hemorrhagic disease virus, bluetongue virus

Introduction

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are small flies, less than 3 mm in length, that can transmit pathogens to humans, wildlife, and domestic animals worldwide. The most economically important viruses transmitted by *Culicoides* are members of the family Reoviridae, genus Orbivirus (Ruder et al. 2015). In North America, the 2 orbiviruses impactful to livestock and wildlife are epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV). These viruses cause severe morbidity and mortality in sheep and cattle, and farmed wild ruminants such as white-tailed deer (*Odocoileus virginianus*) (McVey et al. 2015, Ruder et al. 2015), and increasingly in free-ranging populations of white-tailed deer (Christensen et al. 2020). Although the primary vector species, *Culicoides sonorensis* Wirth and Jones, occurs throughout much of the United States, this species is not common in Florida. EHDV outbreaks in farmed white-tailed deer in Florida have prompted interest in the incrimination of competent vector species in Florida (McGregor et al. 2018, 2019a, Sloyer et al. 2019a, Sloyer and Burkett-Cadena 2021). While *Culicoides insignis* Lutz is a modestly competent EHDV vector in Florida (Cauvin et al. 2019, McGregor et al. 2021a), this species is uncommon in northern Florida, where most EHDV transmission occurs. Two putative EHDV vector species, *Culicoides venustus* Hoffman, and *Culicoides stellifer* (Coquillett) satisfy 3 of the 4 World Health Organization criteria

for incriminating vector species and are common in northern Florida (Barnett 1960, McGregor et al. 2018, 2019a, 2019b).

There are over 300 captive white-tailed deer farms in Florida, contributing substantially to the state economy (Outlaw et al. 2017). The purpose for maintaining a deer farm may vary, with most farms used for breeding of trophy stock, or recreational hunting (Drew 1992, Outlaw et al. 2017, Harmon et al. 2020). Regardless of the purpose, farmed white-tailed deer are more susceptible than wild deer to many vector-borne diseases, particularly EHDV (Cauvin et al. 2019). Several factors may contribute to high infection rates, including exposure to vector arthropods, as well as genetic or environmental factors that increase host susceptibility to disease (Cauvin et al. 2019, McGregor et al. 2021b).

As *Culicoides* species are important vectors of viral pathogens in North America, it is critical to understand the host associations of the nearly 150 species that occur throughout the continent (Borkent and Grogan 2009). At present, only 8 studies have documented host associations of *Culicoides* spp. via bloodmeal analysis in North America (Jorgensen 1969, Tempelis and Nelson 1971, Hopken et al. 2017, McGregor et al. 2018, 2019b, 2021b, Sloyer et al. 2019a). Early bloodmeal analyses of *Culicoides* spp. relied on the precipitin methods, which permitted the identification of a limited set of potential hosts for which reacting antisera were available. Using this method, Jorgensen (1969) found that *Culicoides variipennis* complex members in Washington state fed primarily on horses and swine, with a few bloodmeals from cattle, while Tempelis and Nelson (1971) found that *C. variipennis* complex members in California pastures fed heavily on cattle and rabbits, with a small number of bloodmeals from dogs and horses. More recently, bloodmeal analyses using PCR and Sanger sequencing have been used to quantify diverse patterns of host use for *Culicoides* spp.; unlike precipitin methods, PCR is not limited to a specific set of hosts (Kent 2009). Using PCR-based bloodmeal analyses, investigators have shown that members of the *C. variipennis* complex, though mammalophilic, will readily feed on other host classes, including birds (Hopken et al. 2017). Hopken et al. (2017) was among the first to report on host use of species outside of the *C. variipennis* complex, broadening our understanding of host associations of *Culicoides* spp. in North America. Subsequent studies on *Culicoides* spp. host associations in Florida found similar results, demonstrating that while some *Culicoides* spp. appear to have a proclivity for large mammals, others feed upon multiple classes of vertebrates. Several species, including *Culicoides arboricola* Root and Hoffman, *Culicoides crepuscularis* Malloch, *Culicoides edeni* Wirth and Blanton, and *Culicoides haematopotus* Malloch feed almost exclusively on birds (McGregor et al. 2019a, 2021b, Sloyer et al. 2019a). However, host associations for the majority of *Culicoides* spp. in North America remain undocumented.

The present study substantially expands our understanding of *Culicoides* host associations by contributing over 3,000 species-level host bloodmeal identifications of 18 *Culicoides* spp. sampled from 8 deer farms throughout Florida. The objectives were (i) document the *Culicoides* species that bite white-tailed deer and other potential vertebrate hosts of Orbiviruses on Florida deer farms; (ii) compare the host associations of midge species at farms with and without cattle and/or exotic game; (iii) investigate whether host associations are affected by changes in host availability; and (iv) determine the relative preference for feeding on white-tailed deer. We also compared the host associations of putative EHDV vector species *C. insignis* and *C. stellifer* to determine whether the host use of these species changes across farms. Results of this study will allow deer farmers to understand which *Culicoides* species bite deer and are therefore more likely to transmit EHDV, information that can be used to target control for specific species by selectively treating and mitigating known larval habitats.

Methods

Trapping Locations and Sampling

Biting midges were sampled at 8 deer farms spread throughout northern, central, and southern Florida (Fig. 1). Data from 2 sites in Gadsden County (Gadsden 1 and Gadsden 2, FL), were combined for analyses since few bloodmeals were obtained from these 2 farms. All farms had white-tailed deer, and several farms had other captive game or livestock (Cervidae and/or Bovidae) (Table 1). Many of these sites were sampled in previous studies that compared sampling methods or modeled spatial distribution of *Culicoides* (Sloyer et al. 2018, 2019b). On deer farms that had preserves (Table 1), white-tailed deer roamed freely in a high-fenced landscape of mixed pasture, wetlands, and mixed deciduous/coniferous forest. On 2 farms (Suwannee and Marion), white-tailed deer shared the preserve with diverse exotic bovids and cervids (Table 1), essentially cohabiting the environment.

Culicoides were sampled using CDC miniature light traps over approximately 139 trap nights (Model #: 2836BQ; BioQuip Products, Inc., Rancho Dominguez, CA) with either UV/LED arrays (Model #: 2790V390) or incandescent bulbs, either with or without CO_2 as described in Sloyer et al. (2018). Traps were placed either directly on the outside of deer pens or within pens, approximately 1 m from the ground, except for Marion, where traps were set along paths used by captive white-tailed deer. Midges were captured directly into 95% ethanol, returned to the laboratory and identified to species using morphology (Blanton and Wirth 1979).

Molecular Assays

Engorged *Culicoides* females were processed individually using PCR assays and Sanger sequencing to determine the vertebrate host bitten. Genomic DNA was extracted using InstaGene Matrix (Product #: 7326030; Bio-Rad Laboratories Inc.: Hercules, CA, USA) following the manufacturer protocol. Each 25 µl PCR reaction mixtures consisted of 12.5 µl Platinum Green 2X Master Mix (Invitrogen: Carlsbad, CA, USA), 9 µl molecular-grade water, 0.5 µl forward primer (20 µM), 0.5 µl reverse primer (20 µM), and 2.3 µl extracted bloodmeal PCR product (Sloyer et al. 2019a). Three primer sets targeting all vertebrate classes, previously used for analyzing bloodmeals for mosquitoes and biting midges, were used in the PCR reactions (Blosser et al. 2016, Hoyer et al. 2017, McGregor et al. 2019b, Sloyer et al. 2019a, Stenn et al. 2019). Primer sets targeting specific host vertebrate classes are summarized in Table 2.

Cycling conditions for PCR were identical to those previously published (McGregor et al. 2019b, Sloyer et al. 2019a). Amplified products were run on 1% agarose gel, and amplicons with observable PCR product sequenced and purified using Sanger method by commercial sequencing facility (Eurofins Genomics). GenBank Basic Local Alignment Search Tool (BLASTn) was used to match sequences to the vertebrate host using a 95% sequence similarity as a threshold for positive identification (McGregor et al. 2019b, Sloyer et al. 2019a). Steps were taken to prevent human contamination and included positive and negative control templates in each assay, the use of separate hoods for extraction, mastermix preparation, and addition of template DNA. Finally, all surfaces were disinfected with RNase AWAY (Cat. #: 7000TS1; Thermo Scientific, Waltham, MA) and UV light prior to and following each step.
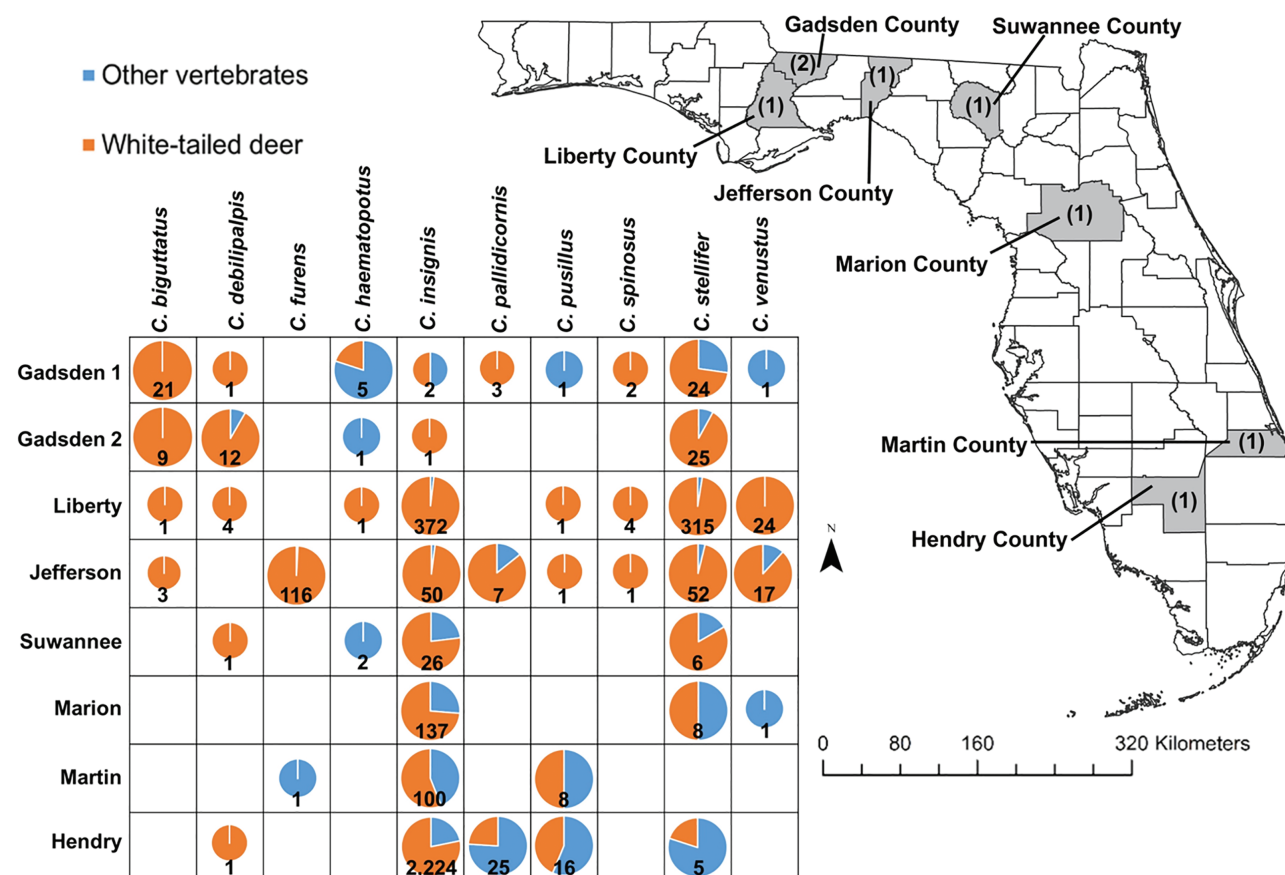
Fig. 1. Host use by *Culicoides* at deer farms in Florida. Pie charts represent bloodmeals of 10 *Culicoides* species from white-tailed deer and all other vertebrates at 8 deer farms. Values represent the number of identified bloodmeals per species at a given farm. Smaller pie charts represent low sample sizes (*n* < 5) for a given species/farm. The map shows the number of farms located in each of 7 Florida counties.

Table 1. Field site (deer farm) descriptors

Study site	Preserve	Size (km²)	Bovids and cervids
Liberty	No	0.80	*Odocoileus virginianus*
Gadsden 1	Yes	2.02	*Odocoileus virginianus*
Gadsden 2	No	0.01	*Odocoileus virginianus*
Jefferson	No	0.20	*Odocoileus virginianus*
Suwannee	Yes	0.20	*Odocoileus virginianus*
			Cervus canadensis
			Cervus nippon
			Antilope cervicapara
			Axis axis
Marion	Yes	8.09	*Odocoileus virginianus*
			Cervus canadensis
			Cervus nippon
			Antilope cervicapara
			Elaphurus davidianus
			Odocoileus virginianus
			Bos taurus
Martin	No	0.01	*Odocoileus virginianus*
Hendry	No	0.01	*Odocoileus virginianus*

Study site was identified by the county name. "Pens" refers to whether some deer were confined to pens, "Preserve" indicates whether some or all animals had access to a free-range area, "Size" indicates the approximate total area of the farm, and Bovid and Cervid species present are listed.

Statistical Analysis

The proportions of bloodmeals which came from white-tailed deer were calculated for each *Culicoides* species at each farm and fit to a binomial generalized linear mixed model using a Bayesian framework (Bolker et al. 2009) in R and Stan (R Development Core Team 2019, Stan Development Team 2022, Guo et al. 2022). In this model, an intercept was included as a fixed effect, while both species and farm were included as random effects. Leave-one-out cross validation was used to compare the predictive performance of this model against alternatives which included fixed effects for the presence of cattle or exotic game on the farms and/or the effect of the farm being adjacent to a reserve. The former was found to improve model fit while the later did not. In all cases, fixed effects were given weakly informative, normal priors with means of 0 and standard deviations of 5, while the standard deviations of the random effects were given half-Cauchy priors with location parameters of 0 and scale parameters of 1 (Gelman et al. 2013). Several *Culicoides* used in the analysis had low bloodmeal sample sizes (*n* < 2 in a pairing), and therefore, little could be concluded about the biting habits of these species from this analysis. These species were *C. arboricola*, *Culicoides bickleyi* Wirth and Hubert, *C. crepuscularis*, *Culicoides floridensis* Beck, *Culicoides hinmani* Khalaf, *Culicoides mississippiensis* Hoffman, *Culicoides paraensis* (Goeldi), and *Culicoides torreyae* Wirth and Blanton.

We analyzed the similarity in host associations of *C. insignis* and *C. stellifer* across farms according to the Morisita-Horn index in the package SpadeR (Chao and Jost 2015, Chao et al. 2016). This analysis helped to determine whether host utilization by these species changes by farm. The Martin site was excluded from the SpadeR calculations as no blood-engorged *C. stellifer* were collected from that site.

Results

White-tailed deer were important hosts for *Culicoides* at deer farms throughout Florida, constituting a high percentage of total bloodmeals at all farms (Figs. 1 and 2). All *Culicoides* species with greater than 5 bloodmeals fed upon white-tailed deer, including established ornithophagic species, such as *C. haematopotus* at some sites (Fig. 1). White-tailed deer constituted a very high percentage (86–100%) of total bloodmeals of *Culicoides biguttatus* (Coquillett), *Culicoides debilipalpis* Lutz, *Culicoides furens* (Poey), and *Culicoides pallidicornis* Keiffer (Fig. 1). When present, livestock (cattle, horses, pigs) and exotic game animals (bovids and cervids) were bitten by *Culicoides* spp. and contributed 20–40% of total bloodmeals (Fig. 2). A wide diversity of livestock and exotic game were bitten, including cattle (*Bos taurus*), horses (*Equus caballus*), wild boar (*Sus scrofa*), elk (*Cervus elaphus*), Sika deer (*Cervus nippon*), axis deer (*Axis axis*), blackbuck (*Antilope cervicapra*), Père David's deer (*Elaphurus davidianus*), Scimitar oryx (*Oryx dammah*), Cape bushbuck (*Tragelaphus sylvaticus*), fallow deer (*Dama dama*), greater kudu and (*Tragelaphus strepsiceros*) (Supplementary Table 1). In total, we analyzed 3,652 blood-engorged *Culicoides* for this study.

Results of the binomial generalized linear mixed model showed apparent differences in host selection between *Culicoides* species, despite the dominant hosts available being white-tailed deer. Although nearly all species analyzed fed on white-tailed deer, the probability of feeding upon white-tailed deer depended on the availability of cattle, or exotic game either directly on the property or on adjacent lands (Fig. 3). When no cattle and/or exotic game were present on farms, nearly all *Culicoides* spp. present were estimated to have a >0.75 probability of feeding on white-tailed deer. The only species with a probability < 0.75, was *C. haematopotus* (estimated probability = 0.61 when no other ungulate species were present) (Fig. 3). The presence of cattle and/or exotic game nearby influenced the feeding habits of *Culicoides* species by decreasing the probability of feeding on a white-tailed deer to ranges of 0.12–0.73, suggesting that cattle and exotic game draw some *Culicoides* spp. away from biting white-tailed deer (Fig. 3).

In general, white-tailed deer constituted a larger percentage of the bloodmeals of *C. stellifer* and *C. insignis* at deer farms in northern Florida than in southern Florida (Fig. 1). At deer farms in northern Florida (Liberty and Jefferson), white-tailed deer contributed 88–100% of bloodmeals of both species. At deer farms

Table 2. Primers used to amplify vertebrate host DNA from *Culicoides* species collected at Florida deer farms

| Primer pair (F/R)	Sequence	Amplicon size (bp)	Target gene	Citation
L2513/	5′-GCCTGTTTACCAAAAACATCAC-3′	244	16S rRNA	Kitano et al. (2007)
H2714	5′-CTCCATAGGGTCTTCTCGTCTT-3′			
L0/H1	5′-GGACAAATATCATTCTGAGG-3′	220	cyt b	Lee et al. (2008)
	5′-GGGTGGAATGGGATTTTGTC-3′			
16L1/	5′-CTGACCGTGCAAAGGTAGCGTAATCACT-3′	450	16S rRNA	Hass et al. (1993); Vidal et al. (2000)
H3056	5′-CTCCGGTCTGAACTCAGATCACGTAGG-3′			
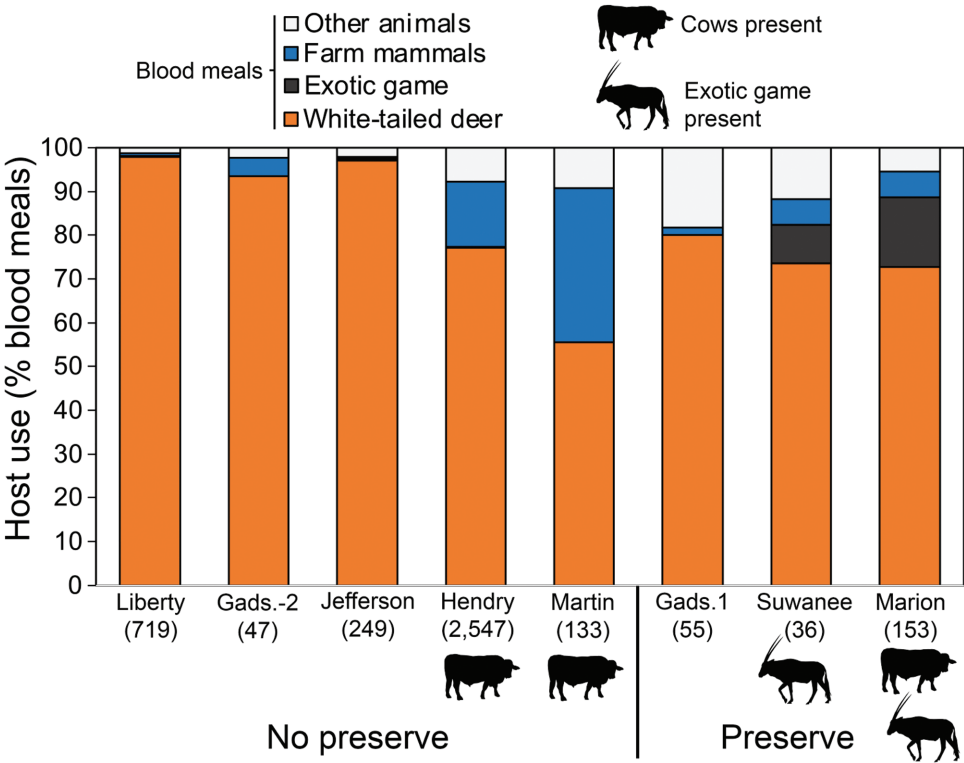
Fig. 2. Host use of *Culicoides* on Florida deer farms. Bars represent percentages of bloodmeals taken from farmed white-tailed deer, exotic game (bovids and cervids), other farm animals (cattle, horse, pig), and other animals (wild mammals, dogs, birds, and reptiles) at each farm by all *Culicoides* spp.

in Marion, Hendry, and Martin (central and southern Florida) white-tailed deer rarely contributed more than 75% of both *C. insignis* and *C. stellifer* bloodmeals (Fig. 1). Apart from white-tailed deer, *C. insignis* and *C. stellifer* fed upon other mammals and reptiles, but not birds or amphibians. Cattle constituted the second highest percent of bloodmeals by *C. insignis* at 9.9% of total bloodmeals (288 of 2,912) (Table 3). This varied by farm however, with cattle constituting a higher percentage of *C. insignis* bloodmeals at the southern sites of Hendry (11.7%) and Martin (22%), whereas at sites north of Martin, cows contributed less than 1% of *C. insignis* bloodmeals. Humans were the third most fed upon vertebrate in the study, comprising 5.7% of total bloodmeals, with the highest percentage (6.9%) observed at Hendry. Horses contributed 2% of *C. insignis* bloodmeals at Hendry and 11% at Martin. Both *C. insignis* and *C. stellifer* bit introduced game animals. For example, at the Liberty site, <1% of bloodmeals of the 2 *Culicoides* species were derived from non-native ungulates,

yet at the Marion site, 15.9% of bloodmeals from *C. insignis* and *C. stellifer* came from non-native ungulates (Table 3). Reptile bloodmeals comprised <1% of total bloodmeals at the Hendry and Liberty sites, but accounted for 16.7% of the bloodmeals at Suwannee, where *C. stellifer* fed upon a brown anole (*Anolis sagrei*) (*n* = 1) (Table 3). *Culicoides insignis* and *C. stellifer* fed on brown basilisk (*Basiliscus vittatus*) at Hendry (*n* = 4) and Liberty (*n* = 1), respectively.

Host use by *C. insignis* was more similar across farms than that of *C. stellifer*. Both host similarity (Morisita-Horn index) and numbers of shared host species were higher for *C. insignis* than for *C. stellifer*. *Culicoides insignis* host similarity ranged from 0.800 to 1.000 between sites while that of *C. stellifer* ranged from 0.380 to 1.000 (Table 4). *Culicoides insignis* always shared at least 2 host species between sites (commonly white-tailed deer and either cow or human hosts) and shared as many as 5 species between Hendry and Martin, and Marion (Tables 3 and 4). *Culicoides stellifer* shared only
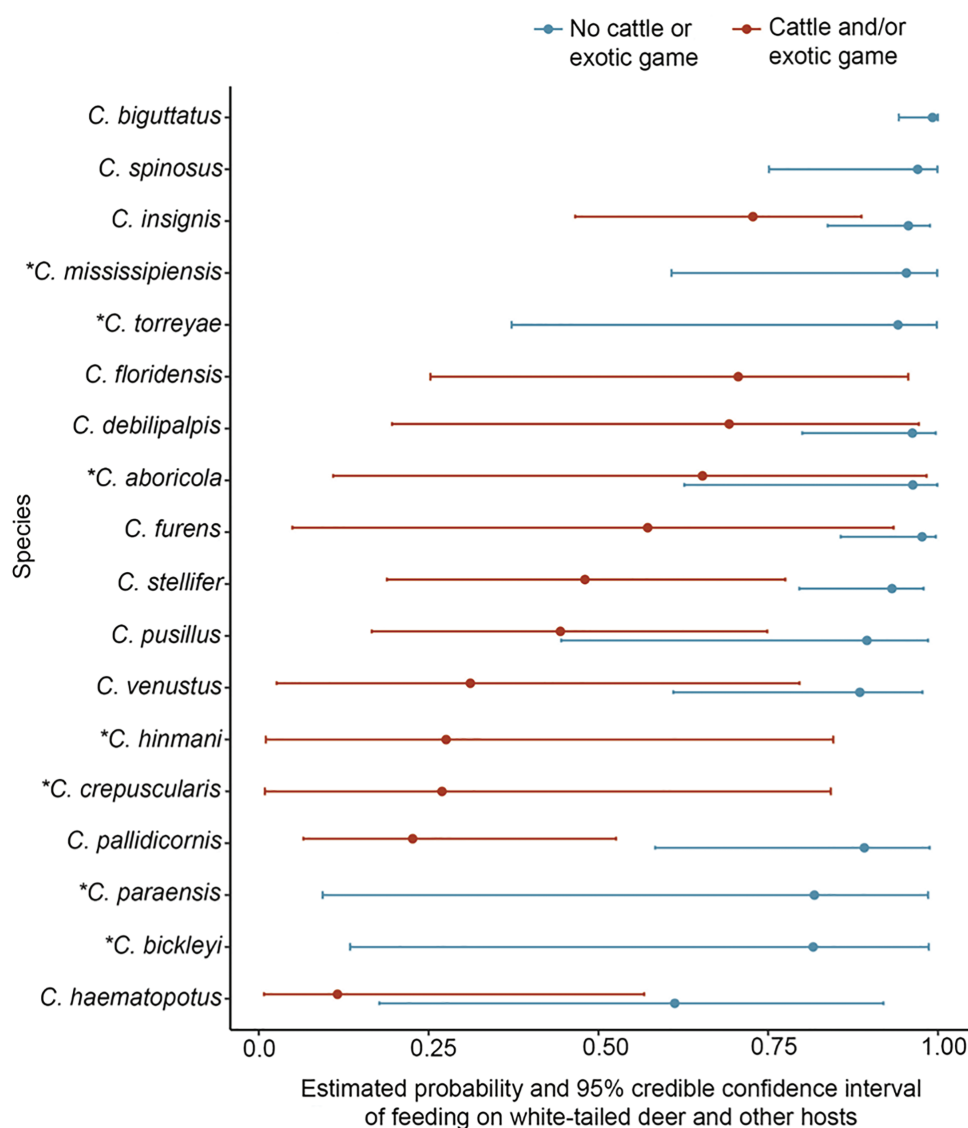

Fig. 3. Estimated probability of *Culicoides* spp. to feed on white-tailed deer at deer farms in Florida. Blue dots and corresponding confidence intervals represent estimation for *Culicoides* spp. bloodmeals collected at deer farms where other ungulate species were not present or directly adjacent to the property. Red dot and corresponding confidence intervals represent the probability that *Culicoides* spp. would feed on white-tailed deer when other ungulate species were nearby. Asterisks denote when a species is represented by <5 bloodmeals.

Table 3. Bloodmeals of *C. insignis* and *C. stellifer* at 8 Florida deer farms

Deer farm	Host class	Host order	Vertebrate host	C. insignis	C. stellifer
Gadsden 1	Mammal	Artiodactyla	*Odocoileus virginianus*	1	16
			Sus scrofa	0	1
		Primates	*Homo sapiens*	1	4
		Rodentia	*Sciurus niger*	0	1
Gadsden 2	Mammal	Artiodactyla	*Odocoileus virginianus*	1	23
			Bos taurus	0	1
			Sus scrofa	0	1
Hendry	Mammal	Artiodactyla	*Odocoileus virginianus*	1,739	1
			Bos taurus	261	1
			Sus scrofa	10	0
			Axis axis	1	0
			Cervus elaphus	0	1
		Carnivora	*Mephitis mephitis*	1	0
			Spilogale putorius	1	0
		Perissodactyla	*Equus caballus*	52	0
		Primates	*Homo sapiens*	153	2
		Rodentia	*Rattus norvegicus*	2	0
	Reptile	Squamata	*Basiliscus vittatus*	4	0
Jefferson	Mammal	Artiodactyla	*Odocoileus virginianus*	49	50
			Cervus elaphus	0	1
		Perissodactyla	*Equus caballus*	0	1
		Primates	*Homo sapiens*	1	0
Liberty	Mammal	Artiodactyla	*Odocoileus virginianus*	365	307
			Sus scrofa	3	2
			Oryx dammah	0	1
		Primates	*Homo sapiens*	3	4
		Rodentia	*Rattus norvegicus*	1	0
	Reptile	Squamata	*Basiliscus vittatus*	0	1
Marion	Mammal	Artiodactyla	*Odocoileus virginianus*	101	4
			Cervus elaphus	8	1
			Antilope cervicapra	4	0
			Tragelaphus strepsiceros	4	0
			Ovis aries	4	0
			Bos taurus	3	0
			Cervus nippon	3	0
			Dama dama	1	0
			Sus scrofa	1	0
			Axis axis	0	1
			Tragelaphus sylvaticus	0	1
		Carnivora	*Procyon lotor*	1	0
		Perissodactyla	*Equus caballus*	1	0
		Primates	*Homo sapiens*	6	1
Martin	Mammal	Artiodactyla	*Odocoileus virginianus*	56	0
			Bos taurus	22	0
			Sus scrofa	2	0
		Perissodactyla	*Equus caballus*	11	0
		Primates	*Homo sapiens*	9	0
Suwannee	Mammal	Artiodactyla	*Odocoileus virginianus*	20	5
			Cervus elaphus	3	0
			Bos taurus	2	0
		Primates	*Homo sapiens*	1	0
	Reptile	Squamata	*Anolis sagrei*	0	1

one host species at several of the deer farms in Florida. At Suwannee for example, *C. stellifer* fed only on white-tailed deer (Tables 3 and 4), and so only shared this species at all other farms in Florida.

Other *Culicoides* fed upon mammals, birds, and reptiles. *Culicoides pallidicornis* (*n* = 35) took 31.4% of bloodmeals from cattle, 32% from human, and 24% from white-tailed deer. *Culicoides pusillus* (*n* = 27) took 25.9% of bloodmeals from cows, 7.4% from horse, 22.2% from human, and 44.4% from white-tailed

deer (Supplementary Table 1). *Culicoides haematopotus* (*n* = 9), considered an ornithophagic species, took 22.2% of bloodmeals from white-tailed deer, 22.2% from human, 11.1% from red-eyed vireo, 33.3% from northern cardinal, and 11.1% from tufted titmouse (Supplementary Table 1). The 4 species that did not feed on white-tailed deer were *C. paraensis* (human = 1), *C. bickleyi* (human, *n* = 1), *C. crepuscularis* (human, *n* = 1), and *C. hinmani* (eastern gray squirrel, *n* = 1) (Supplementary Table 1).

Discussion

This study demonstrates that diverse assemblages of *Culicoides* are active at deer farms in Florida, and most of the species feed on white-tailed deer. Approximately 50 species of *Culicoides* are recorded from Florida (Blanton and Wirth 1979, Grogan Jr et al. 2010, Vigil et al. 2014). Fourteen of 18 *Culicoides* species fed upon white-tailed deer to varying degrees in this study, and the four species that did not feed on white-tailed deer had very low (*n* = 1) sample sizes (*C. hinmani*, *C. crepuscularis*, *C. paraensis*, and *C. bickleyi*). The high diversity of *Culicoides* species feeding upon white-tailed deer suggests that transmission of Orbiviruses on deer farms in Florida is a complex issue, and control of vectors will be complicated by diverse larval ecologies. Our data also indicate that farm location, and the presence of diverse vertebrate hosts both within farms and in adjacent properties, influences host use patterns.

Our findings demonstrate that certain *Culicoides* spp. will actively seek out white-tailed deer even when other ungulate hosts are present, while other species appear to feed on white-tailed deer only when few other host species are available. From this, we infer that while some species may be mammal-feeding generalists, a few appear to actively seek bloodmeals from white-tailed deer, even when other large ungulate species are present. Nonetheless, when cattle and/or exotic game were available, (Fig. 3) some *Culicoides* spp. such as *C. insignis*, and *C. debilipalpis* rarely varied their feeding upon white-tailed deer (Fig. 3), indicating that these 2 species may feed preferentially upon white-tailed deer. This finding corroborates with McGregor et al. (2019b) which showed that *C. debilipalpis* preferred to feed on white-tailed deer. Other species, including *C. biguttatus and C. spinosus* were found only at farms with no cattle or exotic game present and bloodmeals accordingly were found to be from only white-tailed deer, supporting the theory that hosts may influence the spatial distribution of vector species (Burkett-Cadena et al. 2013). The presence of *C. biguttatus* at farms without cattle or exotic game nearby, along with the finding that all 34 bloodmeals were derived from white-tailed deer, corroborates the finding from McGregor et al. (2019b) that *C. biguttatus* prefers white-tailed deer, and also with Hopken et al. (2017) which found that *C. biguttatus* fed solely on white-tailed deer in New York (*n* = 9). Only one species, *C. haematopotus,* did not have a proclivity for feeding upon white-tailed deer, with a predicted probability of 0.61 for feeding on white-tailed deer when no cattle or exotic game were present.

This is very low considering the availability of white-tailed deer. This finding is unsurprising however, given that *C. haematopotus* is a vector of *Haemoproteus* spp. which are avian blood parasites (Atkinson 1988, Garvin and Greiner 2003) and have been found to feed primarily on birds in other studies (McGregor et al. 2019b, Sloyer et al. 2019a).

A few species appeared opportunistic in regard to host use. This was made apparent by the wide differences in estimated probabilities for *C. furens*, *C. stellifer*, *C. pusillus*, *C. venustus*, and *C. pallidicornis* (between 0.41 and 0.66) for feeding on white-tailed deer at farms with no cattle or exotic game and farms with cattle or exotic game present (Fig. 3). This difference generally aligns with findings by McGregor et al. (2019b) which found that *C. stellifer* fed on diverse hosts, with marginal preference for white-tailed deer. *Culicoides furens* is primarily considered a human pest but has been found to take bloodmeals from humans, coyote (*Canis latrans*), and cows (Sloyer et al. 2019a) and is attracted to both avian and mammalian hosts (Hair and Turner 1968, Koch and Axtell 1979). That *C. venustus* and *C. stellifer* may be opportunistic in regard to host use is significant because these species are the most strongly implicated vectors of EHDV in Florida, both having satisfied 3 of the 4 WHO criteria for incriminating vector species (McGregor et al. 2019a, 2019b). The existence of 2 opportunistic vector species, with some proclivity for feeding on white-tailed deer, indicates that these species may serve as bridge vectors for both EHDV and BTV, between native and nonnative mammals or farmed vs. free-ranging white-tailed deer.

The finding that *C. stellifer* had relatively low host similarities between sites when compared to *C. insignis*, further suggests that this species is innately more opportunistic, and as a consequence, may also serve as a bridge vector between nonnative cervids or bovids that are present on some properties. We found that *C. stellifer* feeds primarily on white-tailed deer at farms where only deer are farmed, but readily feed on other hosts, particularly nonnative ungulates (including elk) or wild boar (Table 3), when these animals are present. That *C. stellifer* readily feeds on a variety of hosts has implications for transmission of EHDV between non-native amplification hosts and farmed white-tailed deer. In Florida, elk have been found to be common amplification hosts of EHDV (Cauvin et al. 2019) and are fed upon by *C. stellifer* (McGregor et al. 2019b). Since *C. stellifer* feeds on both elk and white-tailed deer, it may transmit Orbiviruses between these 2 host species, thereby contributing to higher rates of

Table 4. Host use similarity between farms for vector *Culicoides*

Species	Farm	Hendry	Martin	Marion	Suwannee	Jefferson	Gadsden 1 + Gadsden 2	Liberty
C. insignis	Hendry	–	0.939	0.989	0.999	0.965	1.000	0.964
	Martin	5	–	0.915	0.927	0.824	1.000	0.822
	Marion	5	5	–	1.000	0.960	1.000	0.958
	Suwannee	3	3	4	–	0.969	1.000	0.968
	Jefferson	2	2	2	2	–	1.000	1.000
	Gadsden 1 + Gadsden 2	2	2	2	2	2	–	1.000
	Liberty	4	3	3	2	2	2	–
C. stellifer	Hendry	–		1.000	0.435	0.383	0.676	0.380
	Marion	3		–	0.946	0.849	1.000	0.838
	Suwannee	1		1	–	1.000	1.000	1.000
	Jefferson	2		2	1	–	0.952	1.000
	Gadsden 1 + Gadsden 2	2		2	1	1	–	0.951
	Liberty	2		2	1	1	3	–

Results of the Morisita-Horn similarity index for host utilization of *C. insignis* and *C. stellifer* between sites. Cells in the bottom left represent the number of shared host species from bloodmeals between sites while cells on the top right of each species represent similarity of host species from bloodmeals between sites.

EHDV infection among farmed white-tailed deer in Florida (Cauvin et al. 2019). This situation has been documented in Europe, whereby vector *Culicoides*, including *Culicoides imicola* Kieffer, *Culicoides obsoletus* (Meigen), *Culicoides scoticus* Downes & Kettle, *Culicoides pulicaris* L., and *Culicoides punctatus* (Meigen), fed not only on wild ruminants with high seroprevalence for BTV and Schmallenberg virus, but also on domestic ruminants (Talavera et al. 2018). In contrast, species such as *C. venustus*, which fed only on white-tailed deer and humans in this study, may act as a primary vector of EHDV between white-tailed deer (McGregor et al. 2019b). Co-occurrence of multiple vector species with diverse patterns of host use on Florida deer farms likely increases EHDV transmission rates, posing a challenge for vector control efforts.

The finding that *C. insignis* had high host similarity and high numbers of shared species between sites indicates a rather strict innate pattern of host preference for this species. *Culicoides insignis* fed primarily on white-tailed deer at northern farms (Fig. 1) but took a proportion of bloodmeals from other vertebrates, primarily cows, in southern Florida (Fig. 1, Table 3); however, this difference was narrow (Fig. 3). Bloodmeals from cows were most common at Martin (22.0%), and the southernmost site, Hendry (11.7%), where cattle farms abutted the white-tailed deer farms (Table 3), suggesting that when available, cows are readily bitten by *C. insignis*. By feeding upon both white-tailed deer and cows in southern Florida, *C. insignis* is very likely transmitting BTV between farmed bovids and cervids, especially where deer farms and cattle ranches are in close proximity. Our study contains the most extensive documentation of host use of *C. insignis* to date, as only 2 prior studies reported on *C. insignis* host use via bloodmeal analysis (*n* = 6 total bloodmeal identifications) (McGregor et al. 2019b, Sloyer et al. 2019a). In laboratory studies, Florida populations of *C. insignis* were weakly competent vectors of EHDV-2 (Tanya et al. 1992, McGregor et al. 2021a), which could be important as this species expands its range northward (Vigil et al. 2018).

We analyzed one blood-engorged specimen each for *C. paraensis*, *C. hinmani*, *C. crepuscularis*, and *C. bickleyi* (Supplementary Table 1), none of which fed on white-tailed deer. Due to the very low sample sizes for these species, we can draw few conclusions on the host associations of these species. Previous studies indicate that these four species have a range of hosts, including humans, small mammals, and birds (Atkinson 1988, Garvin and Greiner 2003, Hopken et al. 2017, McGregor et al. 2019b, 2021b, Sloyer et al. 2019a), but not white-tailed deer or other ruminants. Therefore, these species are not likely vectors of EHDV or BTV in Florida.

White-tailed deer were hosts for 14 *Culicoides* spp. at deer farms in Florida, indicating that transmission of EHDV and BTV in Florida may not be limited to the small number of *Culicoides* species which have already been incriminated as vectors. This notion is supported by previous work (McGregor et al. 2019b) which documents several species including *C. biguttatus*, *C. debilipalpis*, and *C. pallidicornis* biting white-tailed deer (McGregor et al. 2019b). Larval ecology of these species is very diverse, with some species, such as *C. debilipalpis*, developing in treeholes (Ronderos et al. 2010), and others, such as *C. stellifer*, in extensive wet seepages (Erram et al. 2019, Black et al. 2022). *Culicoides biguttatus*, *C. debilipalpis*, and *C. pallidicornis* have been found to meet 2 vector incrimination criteria, including (i) demonstration that they bite the hosts of EHDV, white-tailed deer (McGregor et al. 2018), and (ii) their temporal and spatial distributions overlap with infection of EHDV in white-tailed deer. However, of these species, only *C. stellifer* has been found to be infected with EHDV at this site (McGregor et al. 2019a). Additionally, at present, only *C. insignis* has been experimentally evaluated for vector competence of EHDV-2 in Florida (McGregor et al. 2021a). As such, further evaluation of field caught *Culicoides* spp. for the detection of BTV and EHDV serotypes, followed by vector competence studies, will be necessary to incriminate more species in these systems.

This study provides valuable quantitative data on the host associations of *Culicoides* species at 8 Florida deer farms from the panhandle to the southern peninsula and investigates how host availability influences the host-feeding patterns. *Culicoides* species present at deer farms feed heavily upon white-tailed deer, but also bite available large mammals, including exotic game. The putative EHDV vector species, *C. stellifer*, was more labile in hosts utilized across farms than *C. venustus*, which had high host similarity between farms. Other species which fed heavily upon deer (*C. furens*, *C. biguttatus*, *C. debilipalpis*, *C. pallidicornis*, and *C. spinous*) warrant further examination as potential Orbivirus vectors. Further field and laboratory studies are needed to fully incriminate (or exonerate) these species, in order to better focus control efforts.

Acknowledgments

Supplementary Material

Supplementary material is available at *Journal of Medical Entomology* online.

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