MONITORING OF CULICOIDES SPP. AT A SITE ENZOOTIC FOR HEMORRHAGIC DISEASE IN WHITE-TAILED DEER IN GEORGIA, USA

Authors: Smith, Kirk E., Stallknecht, David E., Sewell, Christopher T., Rollor, Edward A., Mullen, Gary R., et al.

Source: Journal of Wildlife Diseases, 32(4) : 627-642

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-32.4.627

Journal of Wildlife Diseases, 32(4), 1996, pp. 627–642

MONITORING OF *CULICOIDES* SPP. AT A SITE ENZOOTIC FOR HEMORRHAGIC DISEASE IN WHITE-TAILED DEER IN GEORGIA, USA

Kirk E. Smith,[1] David E. Stallknecht,[1] Christopher T. Sewell,[1] Edward A. Rollor,[1] Gary R. Mullen,[2] and Renee R. Anderson[2]

[1] Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA

[2] Department of Entomology, Auburn University, Auburn University, Alabama 36849-5413, USA

ABSTRACT: Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) were monitored at a Georgia (USA) site where epizootic hemorrhagic disease (EHD) and bluetongue (BT) viruses are enzootic among white-tailed deer (*Odocoileus virginianus*). Collections were made using a captive white-tailed deer and light traps from June 1993 through November 1994. We collected 210,482 females from the captive deer during morning and evening periods. Predominant species were *C. lahillei* (73%), *C. stellifer* (16%), *C. biguttatus* (6%), *C. niger* (3%), *C. spinosus* (2%), and *C. paraensis* (0.2%). Other species were *C. venustus, C. obsoletus/sanguisuga, C. haematopotus, C. guttipennis,* and *C. arboricola,* which together represented <0.1% of the specimens collected. No *C. variipennis,* a known vector of EHD and BT viruses, were collected from the deer. An estimated 953,299 females were collected in 695 light-trap nights. The most common species in light-trap collections were *C. spinosus* (45%), *C. biguttatus* (27%) and *C. stellifer* (24%). *Culicoides variipennis* was rare in the light-trap samples, representing <0.01% of the total collections. There was serological evidence from hunter-killed deer that local deer were infected with EHD and BT viruses during the study, particularly during 1994. A primary suspect vector was *C. lahillei,* which attacked the bait deer in large numbers during the summer and early fall of both 1993 and 1994. Based on their seasonality, relative abundance, and host-seeking activity, *C. stellifer* and *C. spinosus* also were considered as possible vectors. However, virus isolation attempts on 113,716 *Culicoides,* including 62,530 *C. lahillei* and 32,769 *C. stellifer,* were negative.

Key words: *Culicoides* spp., white-tailed deer, *Odocoileus virginianus,* hemorrhagic disease, epizootic hemorrhagic disease virus, bluetongue virus, Georgia, epizootiology.

INTRODUCTION

Infections of white-tailed deer (*Odocoileus virginianus*) with viruses in the epizootic hemorrhagic disease (EHD) or bluetongue (BT) virus serogroups (Reoviridae: Orbivirus) can be inapparent or can lead to a disease syndrome known as hemorrhagic disease (HD) (Prestwood et al., 1974; Thomas, 1981). Hemorrhagic disease is the most important infectious disease affecting white-tailed deer in the United States (Nettles and Stallknecht, 1992).

The vectors of EHD and BT viruses are biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae). *Culicoides variipennis* is considered the primary vector of BT viruses for domestic ruminants in most of the United States and also is a confirmed vector of EHD viruses (Gibbs and Greiner, 1989). *Culicoides variipennis* was implicated as the vector during an epizootic of hemorrhagic disease among captive white-tailed deer in Kentucky (USA) (Jones et al., 1977) and subsequently was demonstrated to be capable of serial biologic transmission of EHD viruses among white-tailed deer (Foster et al., 1977). *Culicoides insignis* is the only other confirmed vector of BT viruses in the United States, but this has been demonstrated only for domestic ruminants in Florida (USA) (Tanya et al., 1992).

Culicoides spp. other than *C. variipennis* and *C. insignis* also may act as vectors of EHD or BT viruses in the United States (Jones, 1985; Mullen et al., 1985a; Mullens, 1992). This may be particularly true in geographic areas where *C. variipennis* or *C. insignis* are not the predominant species attacking ruminants or where they are relatively uncommon. These conditions have been reported over much of the

southeastern United States (Tanner and Turner, 1975; Hayes et al., 1984; Mullen et al. 1985a; Gerhardt, 1986; K. E. Smith, unpubl.). Other potential *Culicoides* spp. vectors of BT viruses indigenous to the southeastern United States are *C. lahillei* (= *debilipalpis*), *C. stellifer, C. paraensis, C. obsoletus, C. biguttatus,* and *C. venustus* (Gibbs and Greiner, 1989). However, no *Culicoides* spp., including *C. variipennis* and *C. insignis,* have been demonstrated to be significant vectors of EHD or BT viruses for free-ranging white-tailed deer in the United States.

In the United States, HD occurs most frequently in the Southeast, where it is reported annually (Nettles et al., 1992). Georgia (USA) is one of seven southeastern states where HD is considered enzootic (Nettles et al., 1992). Within these enzootic states, the highest prevalence of antibodies against EHD and BT viruses in white-tailed deer occurs in the Coastal Plain physiographic region (Stallknecht et al., 1991, 1995).

Our objective was to determine which *Culicoides* spp. were the most likely vectors of EHD and BT viruses circulating among white-tailed deer at an enzootic site in the Coastal Plain region of Georgia. This was accomplished by monitoring *Culicoides* spp. populations for 18 mo at a site where HD activity among white-tailed deer has been documented almost annually since 1980 (D. E. Stallknecht, unpubl.). Virus isolation on *Culicoides* spp. was attempted and local virus activity during insect collections was confirmed by serologic tests for EHD and BT virus antibodies in hunter-killed white-tailed deer and nearby cattle.

MATERIALS AND METHODS

Chickasawhatchee Wildlife Management Area (CWMA) is located in the Coastal Plain in Baker, Calhoun, and Dougherty Counties of southwestern Georgia (31°29′N, 84°23′W) and is administered by the Georgia Department of Natural Resources (GDNR). Approximately 54% of CWMA is classified as wetland, predominately stream-associated bottomland hardwoods and cypress ponds. Common overstory flora species include swamp white oak (*Quercus michauxii*), water oak (*Quercus nigra*), swamp blackgum (*Nyssa sylvatica*), swamp tupelo (*Nyssa aquatica*), bald cypress (*Taxodium distichum*), pond cypress (*Taxodium nutans*), and spruce pine (*Pinus glabra*). The remainder of CWMA is upland habitat comprised primarily of stands of slash pine (*Pinus elliottii*) and loblolly pine (*Pinus taeda*) ranging from seedlings to harvest-age trees. The estimated white-tailed deer density on CWMA during 1993 and 1994 was 13.5 deer per km^2 (R. Simpson, GDNR, pers. comm.).

FIGURE 1. Portable cage used to hold deer while collecting *Culicoides*. Bar = 20cm.

Culicoides spp. adults were collected from a captive white-tailed deer from mid-July 1993 through November 1994. The deer was held in a small portable cage (Fig. 1) in a clearing between upland pine and bottomland hardwood habitats in the interior of CWMA. *Culicoides* spp. were collected from the deer by examining the animal every 1 to 2 min and aspirating observed midges with hand-held, battery-powered aspirators (Hausherr's Machine Works, Toms River, New Jersey, USA). Collectors remained approximately 10 m from the deer when not aspirating midges. Collections were made on three consecutive evenings and three consecutive mornings every second week, except during periods of cooler temperatures from December 1993 through February 1994, when collections were made every third week. Morning collections were made from 0.5 hr before sunrise to a minimum of 1.0 hr after sunrise. If *Culicoides* spp. activity continued longer than 1.0 hr after sunrise, morning collections were continued until activity ceased. Evening

collections were made from 1.0 hr before sunset to 0.5 hr after sunset. From December 1993 through February 1994, morning collections were extended to 3 hr after sunrise and evening collections were begun 2 hr before sunset.

Culicoides spp. adults were collected with light traps from June 1993 through November 1994. From June 1993 through November 1993, light traps were operated weekly on an alternating schedule of three consecutive nights 1 wk followed by two consecutive nights the next week. From December 1993 through February 1994, light traps were operated three consecutive nights every third week. From March through November 1994, they were operated three consecutive nights every second week.

All major habitat types were sampled. One New Jersey light trap (Hausherr's Machine Works) was operated at the northeastern corner of CWMA adjacent to a farm with pastured domestic cattle and goats. Five downdraft blacklight traps (John W. Hock Company, Gainesville, Florida) powered by 12-volt batteries were operated in the interior of CWMA at intervals of ≥2 km. Light traps were operated 0.5 to 1.0 hr before sunset to 0.5 to 1.0 hr after sunrise.

For virus isolation attempts, midges were collected into chilled phosphate-buffered saline (PBS) (Nevill et al., 1992) containing 0.1% Triton-X and 100 mg tetracycline (Sigma Chemical Company, St. Louis, Missouri, USA) per liter. For routine identifications, midges were collected into 80% ethanol.

Large insects were removed from light-trap collections by washing the trap contents through a 10-mesh standard sieve with tap water. Contents passing through the sieve were sampled using a system modified after Eve and Kellogg (1977). Briefly, sieved contents were poured into a graduated Erlenmeyer flask and brought up to 1000 ml with tap water. After thorough mixing, two 50 ml (5%) aliquots were removed and stored in 80% ethanol for later examination. The remainder of each light-trap collection was rinsed with tap water and PBS and then stored at 4 C in PBS containing 200,000 units penicillin, 500 mg streptomycin, 100 mg tetracycline, 100 mg gentamycin, and 5 mg amphotericin B (Sigma Chemical Company) per liter or was stored in 80% ethanol if not used for virus isolation.

The number of *Culicoides* spp. in light-trap collections was either determined by total counts or estimated based on aliquot counts. When total counts could not be obtained due to time constraints or large numbers of midges, a 5% aliquot was counted. If the first aliquot contained <500 *Culicoides* spp., the second aliquot also was counted. Of 695 light-trap-nights from 6 June 1993 to 10 November 1994, entire catches were examined for 312 trap-nights, two 5% aliquots were examined for 357 trap-nights, and only one 5% aliquot was examined for 26 trap-nights. For estimation of seasonality and relative abundance, the average number of each *Culicoides* sp. collected per trap-night per week was calculated. A summed mean for each species during a given collection week was calculated by summing the per-night means from each of the six light traps for that week.

Culicoides spp. collected from the captive deer were rinsed with PBS until free of grossly visible contaminants such as hair or dirt. Midges were then stored at 4 C in sterile PBS containing antibiotics as described until sorted.

Culicoides spp. were identified to species by the adult female key, wing patterns, and descriptions in Blanton and Wirth (1979). These included all *Culicoides* spp. in collections from deer and in all 5% aliquots from light-trap collections that were examined. Light-trap material sorted in addition to the aliquots was examined for *C. variipennis, C. stellifer, C. lahillei, C. biguttatus, C. venustus, C. paraensis, C. obsoletus/sanguisuga* and *C. insignis,* whereas other *Culicoides* spp. were identified to species only if time permitted. Because of difficulty in distinguishing females of *C. obsoletus* and *C. sanguisuga,* these two species were grouped together as *C. obsoletus/sanguisuga. Culicoides scanloni/piliferus* were similarly combined. Representative specimens of each commonly collected species have been deposited in the U. S. National Parasite Collection, Smithsonian Institution, Washington, D.C.

Culicoides spp. for virus isolation attempts were identified and counted in refrigerated PBS on an electronic chill table (Miles Laboratories, Inc., Westmont, Illinois, USA). Midges were pooled according to species, sex, and engorgement status (blood-fed vs nonblood-fed). Maximum pool size usually was 100 midges. All pools were stored at 4 C in 2.0-ml plastic vials with 1.3 ml of PBS containing 1,000,000 units penicillin, 500 mg streptomycin, 100 mg tetracycline, 250 mg gentamycin, and 25 mg amphotericin B per liter. Total storage time of *Culicoides* spp. from collection until virus isolation attempts in most cases was 8 to 10 days.

For virus isolation, approximately 0.65 ml of 0.5 mm zirconium beads (Biospec Products, Bartlesville, Oklahoma, USA) was added to each vial of *Culicoides* sp.. Midges were then emulsified with a mini-bead beater (Biospec Products) for 2 to 3 min until finely and uniformly ground. Samples were sonicated and then centrifuged for 15 min at 1,500 × *G*. We

added 200 μl of supernatant of each sample to a suspension of approximately 8×10^4 baby hamster kidney (BHK_{21}) cells (American Type Culture Collection, Rockville, Maryland, USA) in a single well on 24-well tissue culture plates. Inoculated cells were maintained at 33.5 C in a humidified 5% CO_2 atmosphere for 7 days or until cytopathic effect was observed. If no cytopathic effect was observed by 7 days, cells were scraped and reinoculated onto fresh BHK_{21} cells for a second 7-day passage. For use as a positive control, *C. variipennis sonorensis* infected with EHD virus serotype 2 by intrathoracic inoculation 14 days previously were obtained from the United States Department of Agriculture, Agricultural Research Service, Arthropod-borne Animal Diseases Research Laboratory, Laramie, Wyoming (USA). These control flies were stored at 4 C in sterile PBS with antibiotics. Three pools of one to two midges were tested by virus isolation as described; two pools of two midges each were tested after 6 wk and a single midge was tested after 15 wk.

Serum samples were collected during 1989 to 1994 from white-tailed deer killed by hunters at CWMA during October through December. Age of deer was determined by tooth eruption and wear patterns (Severinghaus, 1949). During January 1994, serum samples also were collected from yearling cattle born and raised on a farm adjacent to CWMA. All serum samples were tested for precipitating antibodies to EHD or BT virus serogroups by agar gel immunodiffusion (AGID) (Pearson and Jochim, 1979) using commercial EHD virus and BT virus antibody test kits (Veterinary Diagnostic Technology, Inc., Wheat Ridge, Colorado, USA) following the manufacturer's instructions. Positive samples then were tested by serum neutralization (SN) against all North American serotypes of EHD virus (1 and 2) and BT virus (2, 10, 11, 13, and 17) as described by Stallknecht et al. (1995). Prevalences of precipitating antibodies to EHD or BT viruses were tested for independence to year and age class using chi-square tests (Dean et al., 1994).

Climatological data (daily temperatures and 30-yr departures from normal for monthly precipitation and monthly average temperature) for recording stations near CWMA were obtained from monthly reports of the National Oceanic and Atmospheric Administration (1993, 1994). The recording station nearest the study site (31°32′N, 82°24′W) was the primary location for climatological data. However, daily temperatures for one collection period during June 1994 were not recorded for the primary site and thus were obtained from the next nearest recording station (31°11′N, 84°12′W).

RESULTS

We collected 210,482 *Culicoides* spp. females from the captive deer from 17 July 1993 through 11 November 1994 (Tables 1 and 2). Predominant species were *C. lahillei* (73%), *C. stellifer* (16%), *C. biguttatus* (6%), *C. niger* (3%), and *C. spinosus* (2%). In addition, 24 *C. lahillei* males and two *C. stellifer* males were collected from the deer. No *Culicoides* spp. were captured from the deer during the collection periods 5 to 8 December 1993, or 4 to 7 January, 24 to 27 January, 15 to 18 February, and 7 to 10 March 1994.

Overall, the percentage of *Culicoides* spp. collected from the deer during morning collections, as opposed to evening collections, tended to reflect the average maximum temperature during collection days (Fig. 2). On a species basis, the percentages collected from the deer during morning collections were as follows: *C. lahillei* (94%), *C. stellifer* (40%), *C. biguttatus* (48%), *C. niger* (65%) and *C. spinosus* (62%).

An estimated 965,079 *Culicoides* spp. were collected with light traps, including 953,299 (99%) females. *Culicoides* spp. were rare or absent in light-trap collections made during late November through mid-February. Subsequent data refer to *Culicoides* females unless otherwise noted. Species most commonly collected in light traps were *C. spinosus* (45%), *C. biguttatus* (27%), and *C. stellifer* (24%). Other species consistently collected in lower numbers were *C. haematopotus* (1.8%), *C. venustus* (0.8%), *C. travisi* (0.5%), *C. scanloni/piliferus* (0.2%), *C. lahillei* (0.2%), *C. niger* (0.2%), and *C. crepuscularis* (0.1%). Species infrequently identified in light-trap collections were *C. arboricola, C. baueri, C. bickleyi, C. guttipennis, C. hinmani, C. insignis, C. obsoletus/sanguisuga, C. paraensis, C. variipennis,* and *C. villosipennis.*

Culicoides lahillei was by far the most abundant species collected from the deer, with peak numbers occurring during Sep-

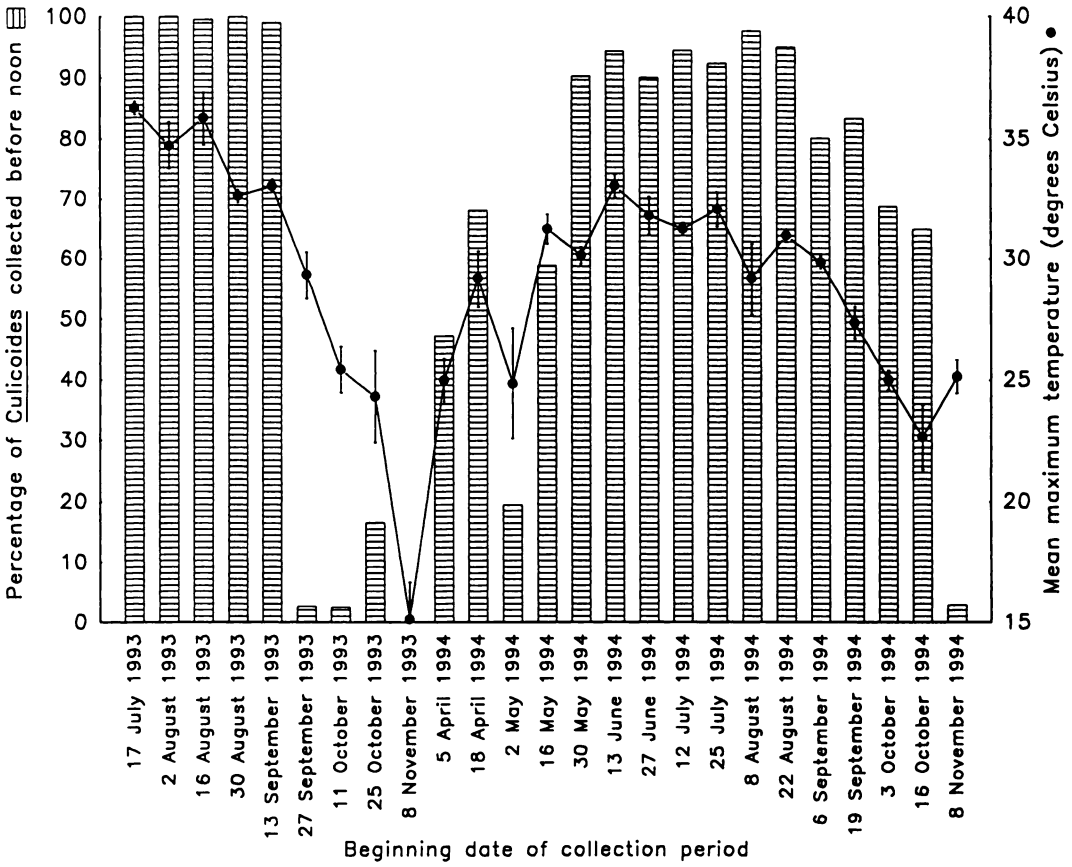

FIGURE 2. Percentage of total daily *Culicoides* spp. collected from deer before noon and mean maximum temperature on collection days. Bars are used to designate standard errors.

tember in 1993 and August in 1994 (Tables 1 and 2). During 13 to 16 September 1993, over 6,700 *C. lahillei* were collected from the deer on each of two mornings. During 1994, large numbers of *C. lahillei* were collected from the deer from June through October (Table 2). Attack rates of 6,038 and 7,294 per morning were ob-

TABLE 1. Total number of *Culicoides* spp. females collected from white-tailed deer during 1993 at Chickasawhatchee Wildlife Management Area, Georgia, by species.

	Collection period[a]										
	July	August			September		October		November		
Species	17	2	16	30	13	27	11	25	8	21	Total
lahillei	264	183	2,896	827	16,648	237	33	117	4	0	21,209
stellifer	6	3	32	22	108	2,318	10,857	927	42	5	14,320
paraensis	0	0	0	7	4	10	28	6	0	0	55
spinosus	0	0	0	0	0	0	2	0	0	0	2
venustus	0	0	0	0	1	0	1	0	0	0	2
guttipennis	0	0	0	0	0	0	2	0	0	0	2
haematopotus	0	0	0	0	0	0	1	0	0	0	1

[a] Each collection period consisted of three consecutive evenings and three consecutive mornings beginning on the evening of the indicated date.

TABLE 2. Total number of *Culicoides* spp. females collected from white-tailed deer during 1994 at Chickasawhatchee Wildlife Management Area, Georgia, by species.

	Collection period[a]																	
	March	April		May			June		July		August		September		October		November	
Species	21	4	18	2	16	30	13	27	12	25	8	22	6	19	3	16	8	Total
lahillei	0	9	241	485	285	189	9,169	16,294	3,796	9,438	25,240	46,414	12,611	5,238	1,855	1,142	14	132,420
stellifer	2	29	119	1,224	409	216	282	183	9	755	680	107	9,012	1,682	2,926	858	385	19,144
biguttatus	0	1,889	4,336	4,247	1,235	22	2	2	0	0	0	0	0	0	0	0	0	11,733
niger	4	27	369	225	2,525	2,434	901	41	0	0	0	0	0	0	0	0	0	6,526
spinosus	0	145	542	409	74	37	6	4	6	117	514	118	2,050	25	404	82	47	4,580
paraensis	0	0	2	0	3	0	7	40	48	53	76	66	54	29	15	7	0	400
venustus	0	0	0	0	2	0	1	1	0	0	3	0	28	0	20	6	0	61
obsoletus/ sanguisuga	0	4	4	6	1	0	0	1	1	1	0	0	0	0	0	0	0	18
haematopotus	0	0	1	1	0	0	0	0	0	3	0	0	0	0	0	0	0	5
arboricola	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
guttipennis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2

[a] Each collection period consisted of three consecutive evenings and three consecutive mornings, beginning on the evening of the indicated date, except for the 13 June period, which included only two evenings and three mornings.

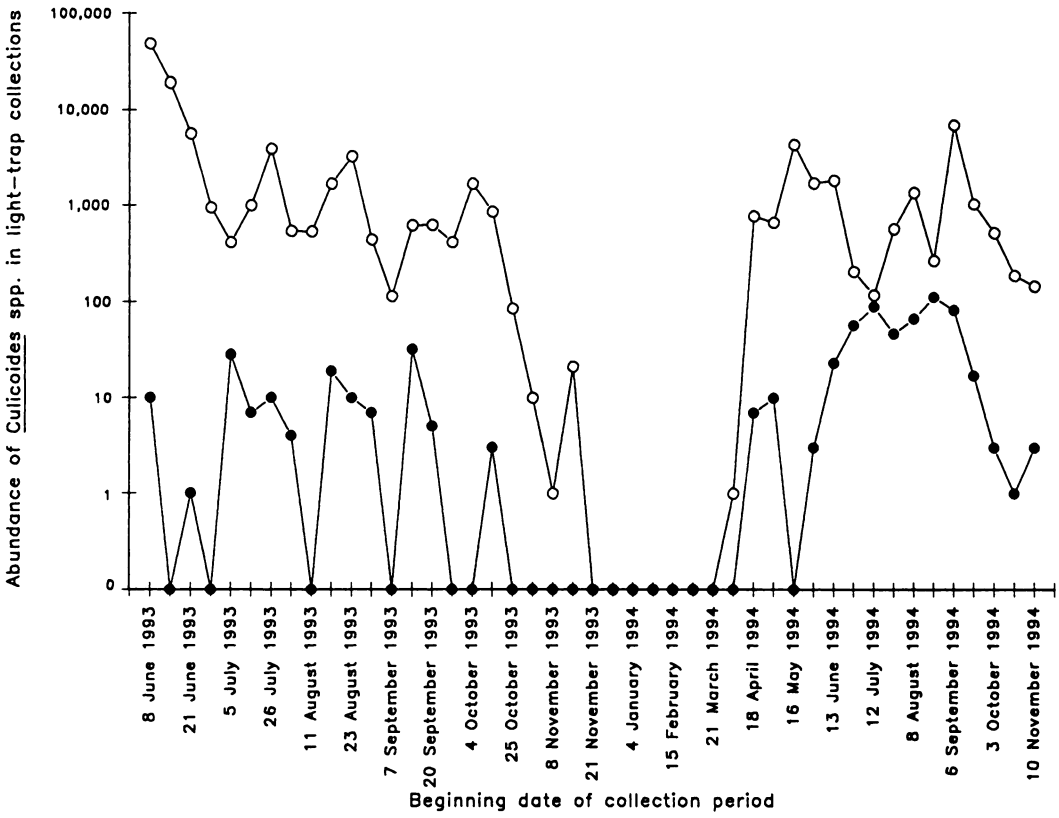

FIGURE 3. Abundance of *C. stellifer* (○) and *C. lahillei* (●) in light trap collections, by collection period. Each collection period usually consisted of two to three nights. The abundance figure used for each period was calculated by summing the per night average of each of six light traps.

served for *C. lahillei* during the 13 to 16 June and 27 to 30 June 1994 periods, respectively. Five of six mornings during August 1994 yielded >10,000 *C. lahillei* per morning; the peak morning was 23 August 1994, when 20,840 *C. lahillei* were collected from the deer. During peak collection periods, peak activity usually occurred from approximately 0.5 to 2 hr after sunrise, and activity commonly lasted until approximately 3 hr after sunrise. The seasonality of *C. lahillei* based on light-trap collections (Fig. 3) coincided well with collections from the deer, although much lower numbers of *C. lahillei* were collected in light traps.

Culicoides stellifer also attacked deer in large numbers, with peak attack rates of 6,423 during the evening of 11 October in 1993 and 4,572 during the morning of 7 September in 1994. *Culicoides stellifer* was by far the most abundant species in light-trap collections during June through October 1993 (Fig. 3). Up to an estimated 24,520 *C. stellifer* were collected per light trap during a single night in early June 1993. Moderate to high numbers of *C. stellifer* were present in light-trap collections from July through October 1993 (Fig. 3). Numbers of *C. stellifer* in 1994 light-trap collections never reached the levels of June 1993. However, numbers collected during summer and fall 1994 were otherwise comparable to numbers during summer and fall 1993 (Fig. 3).

Culicoides biguttatus and *C. niger* both had a distinct spring seasonality based on deer and light-trap collections (Table 2, Fig. 4). Peak catches from the deer were 1,746 during one evening in April 1994 for

TABLE 3. Prevalence of precipitating antibodies to epizootic hemorrhagic disease or bluetongue viruses in white-tailed deer from Chickasawhatchee Wildlife Management Area, Georgia, 1989 to 1994.

Year	Age class of deer (yr)				
	0.5	1.5	2.5	3.5+	Total
1989[a]	3/5 (60)[b]	17/21 (81)	17/17 (100)	6/7 (86)	43/50 (86)
1990[a]	0/3 (0)	3/5 (60)	10/13 (77)	7/7 (100)	20/28 (71)
1991[a]	2/5 (40)	9/10 (90)	10/10 (100)	15/16 (94)	36/41 (88)
1992	2/20 (10)	13/16 (81)	8/9 (89)	36/36 (100)	59/81 (73)
1993	3/16 (19)	5/15 (33)	10/10 (100)	35/35 (100)	53/76 (70)
1994	6/13 (46)	11/15 (73)	12/16 (75)	23/28 (82)	52/72 (72)
Total	16/62 (26)	58/82 (71)	67/75 (89)	122/129 (95)	263/348 (76)

[a] Data for 1989 to 1991 is from Stallknecht et al. (1991).
[b] Number positive/number tested (percent positive).

C. biguttatus and 1,557 during one morning in late May 1994 for *C. niger*. Very high numbers of *C. biguttatus* were present in light-trap collections during April and May 1994, including an estimated 28,540 in one light trap during a single night in April 1994.

Culicoides spinosus was consistently present in collections from deer during 1994, even though it was virtually absent during 1993 (Tables 1 and 2). The largest numbers of *C. spinosus* from the deer were collected during early September 1994 (Table 2), when 948 were collected during one

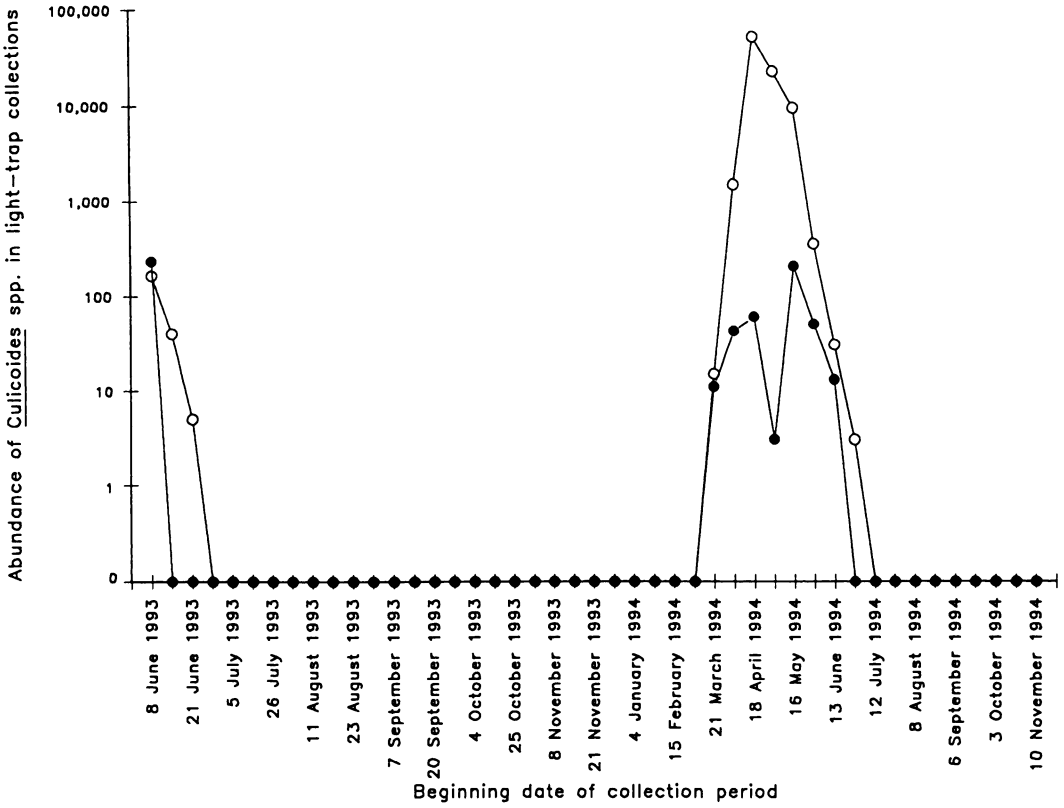

FIGURE 4. Abundance of *C. biguttatus* (○) and *C. niger* (●) in light trap collections, by collection period. See Fig. 3 for explanation.

morning. In 1993, *C. spinosus* was consistently present in light-trap collections only in June (Fig. 5). Very high numbers of *C. spinosus* were collected during April and May 1994 (Fig. 5), including an estimated 36,620 in one light trap during a single night. Low to high numbers of *C. spinosus* were present in light-trap collections during the remainder of 1994, including very high numbers during early August and early September (Fig. 5).

Culicoides paraensis was collected in low numbers from the deer, with larger numbers being collected during 1994 than during corresponding periods in 1993 (Tables 1 and 2). Almost all *C. paraensis* were collected from June through October. *Culicoides paraensis* was rare in light-trap collections; those that were present were collected during June through August (Fig. 6).

Culicoides venustus was collected in low numbers from the deer, primarily during September and October. More were collected during 1994 than during corresponding periods in 1993. *Culicoides venustus* was consistently present in light-trap collections in low to moderate numbers throughout the study except for December 1993 through February 1994 (Fig. 7). Peak light-trap catches occurred during June in 1993 and during September through November in 1994 (Fig. 7).

Culicoides obsoletus/sanguisuga were collected only rarely from the deer, mostly during April and May 1994. These species were present in very low numbers in light-trap collections, primarily during March through August (Fig. 6). *Culicoides haematopotus* was rarely collected from the deer, but was consistently present in light-trap collections in low to moderate numbers during virtually the entire study (Fig. 5).

No *C. variipennis* were present in collections from the deer. *Culicoides variipennis* also was rare in light-trap collections; the total estimated numbers of *C. variipennis* collected during 1993 and 1994 were 83 females and 23 males. Thirty-three females and 20 males were collected in late March 1994; the remainder were represented by individual specimens collected sporadically throughout the study (Fig. 7). Seventeen *C. insignis* (14 females and three males) were present in light-trap collections made during November 1993 ($n = 5$), March 1994 ($n = 1$), October 1994 ($n = 1$), and November 1994 ($n = 10$).

Attempts to isolate virus were conducted on 113,716 *Culicoides* spp. collected at CWMA, including 42,572 from 1993 and 71,144 from 1994. The number tested by species included 62,530 *C. lahillei,* 32,769 *C. stellifer,* 7,969 *C. biguttatus,* 5,744 *C. niger,* 383 *C. venustus,* 120 *C. spinosus,* 60 *C. variipennis,* 24 *C. obsoletus/sanguisuga,* and 4,586 specimens of other *Culicoides* species, including an unknown number of *C. niger* and *C. spinosus.* All specimens tested were females except for 102 *C. stellifer* males, 21 *C. venustus* males, 22 *C. variipennis* males, and 71 males of other *Culicoides* spp.. All CWMA *Culicoides* spp. tested by virus isolation were negative. Epizootic hemorrhagic disease virus serotype 2 was isolated from the two pools of two positive control *C. variipennis* stored at 4 C for 6 wk. The single positive control *C. variipennis* tested after 15 wk of storage was negative.

The overall prevalence of precipitating antibodies to EHD or BT viruses among CWMA deer was significantly ($P < 0.001$) higher in the ≥2.5-yr age classes (93%) than in the 0.5-yr (26%) or 1.5-yr (71%) age classes. Antibody prevalence in deer ≤1.5-yr old was significantly ($P = 0.014$) higher in 1994 (61%) than in 1993 (26%). Antibody prevalence was significantly higher in 1.5-yr-olds in 1991 (90%) versus 0.5-year-olds in 1990 (0%) ($P = 0.014$), in 1.5-yr-olds in 1994 (73%) versus 0.5-yr-olds in 1993 (19%) ($P = 0.007$), and in 2.5-yr-olds in 1994 (75%) versus 1.5-yr-olds in 1993 (33%) ($P = 0.004$). Serologic evidence of exposure of deer to EHD virus serotype 2 (EHDV-2) and BT virus serotype 13 was present each year from 1989 to 1994. Serologic evidence of exposure of

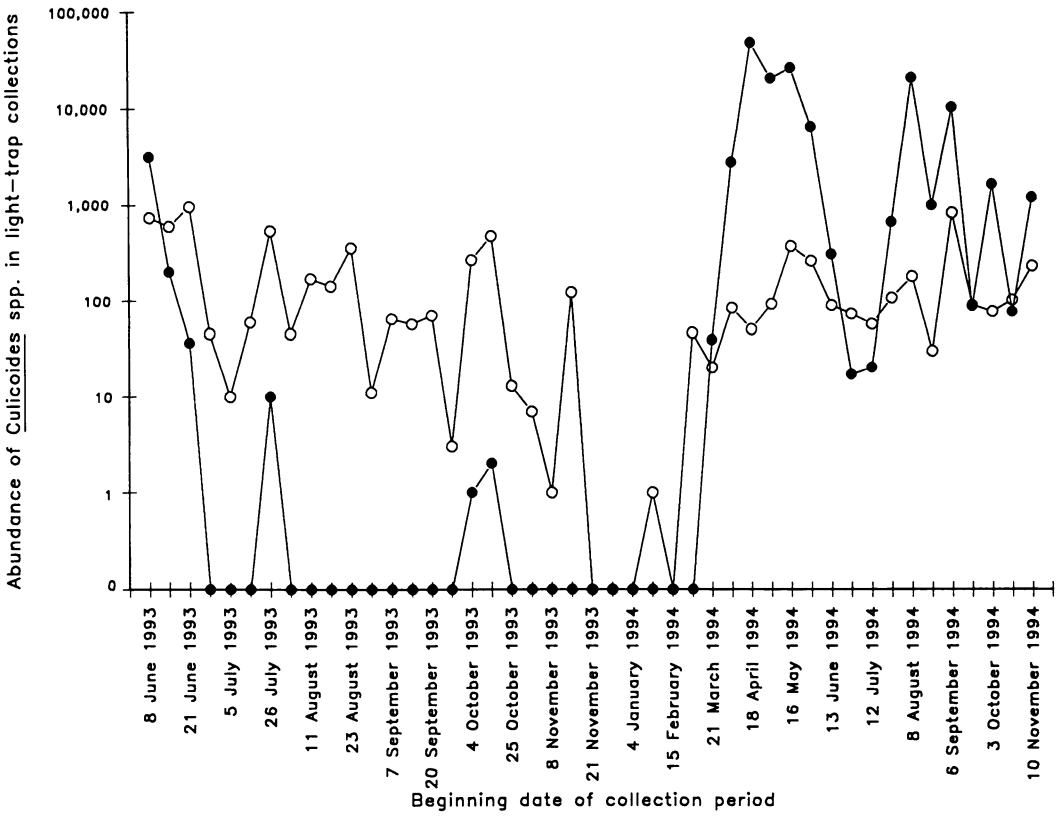

FIGURE 5. Abundance of *C. haematopotus* (○) and *C. spinosus* (●) in light trap collections, by collection period. See Fig. 3 for explanation.

deer to EHD virus serotype 1 (EHDV-1) was present from 1991 through 1993.

Based on serologic data, EHDV-2 was the predominant serotype active during the study. All but one of the 105 AGID-positive deer collected during 1993 and 1994 tested positive for EHDV-2 by SN at a serum dilution of 1:20. Two (8%) of 25 yearling cattle tested in January 1994 were AGID-positive for antibodies to EHD or BT viruses. Serum neutralization testing of the positive cattle sera revealed monospecific reactions to EHDV-2. Relatively few AGID-positive deer were positive for antibodies to BT viruses in 1993 (9.4%) and 1994 (7.7%) by SN at a serum dilution of 1:20. All deer from 1993 and 1994 with SN titers to BT viruses or EHDV-1 were ≥2.5-yr old when killed.

The summer of 1993 in the CWMA area was hotter and dryer than normal, whereas the summer of 1994 was cooler and wetter than normal (Fig. 8).

DISCUSSION

The serologic data are compatible with a pattern of enzootic EHD or BT virus activity in the CWMA white-tailed deer population, as previously described for deer of the Coastal Plain of Georgia (Stallknecht et al., 1991). Additional evidence of enzootic activity at CWMA was provided by an annual mail survey of state wildlife agencies begun in 1980 to monitor HD activity (Nettles et al., 1992, D. E. Stallknecht, unpubl.). Based on survey results, HD activity occurred in the form of chronic lesions in hunter-killed deer from at least one of the three counties represented on CWMA during each year from 1985 to 1994, except for 1993. During our study, it appeared that HD virus activity at

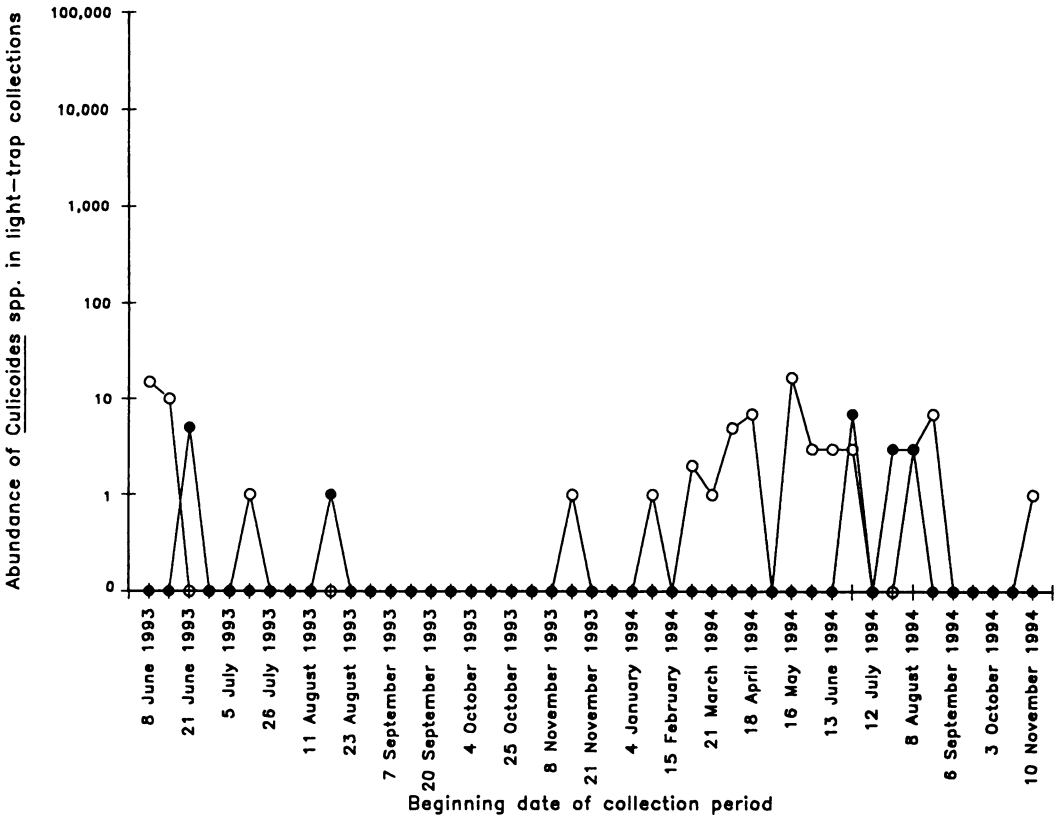

FIGURE 6. Abundance of *C. obsoletus/sanguisuga* (○) and *C. paraensis* (●) in light trap collections, by collection period. See Fig. 3 for explanation.

CWMA was low during 1993 but increased significantly during 1994.

The seasonal periods of virus transmission during the present study are unknown. Peracute or acute clinical cases of HD in white-tailed deer throughout the United States occur primarily in late summer to early fall (Nettles and Stallknecht, 1992). In the southeastern United States, 90% of peracute or acute HD cases diagnosed in white-tailed deer from 1971 to 1980 occurred during August, September, or October; the remainder occurred during June, July, or November (Couvillion et al., 1981). During 1994, peracute and acute HD were frequently diagnosed in captive and free-ranging white-tailed deer throughout much of the southeastern United States (D. E. Stallknecht, unpubl.). Most viral isolates were EHDV-2, with cases observed from late July through early October (D. E. Stallknecht, unpubl.). Thus, in evaluating which *Culicoides* spp. are likely to represent significant vectors of EHD and BT viruses for white-tailed deer at CWMA, it is logical to focus on species that are abundant at least during late July through October.

Culicoides lahillei was considered the primary suspect vector at CWMA based on the tremendous numbers attacking deer, particularly during summer and early fall. *Culicoides lahillei* also was the predominant species collected from deer during two HD epizootics that occurred among captive white-tailed deer in Georgia and North Carolina during 1994 (Smith and Stallknecht, 1996). Replication of EHDV-2 occurs in *C. lahillei*, with up to 8.7% of the midges becoming infected after feeding on viremic white-tailed deer (Smith et al., 1996). There also is evidence

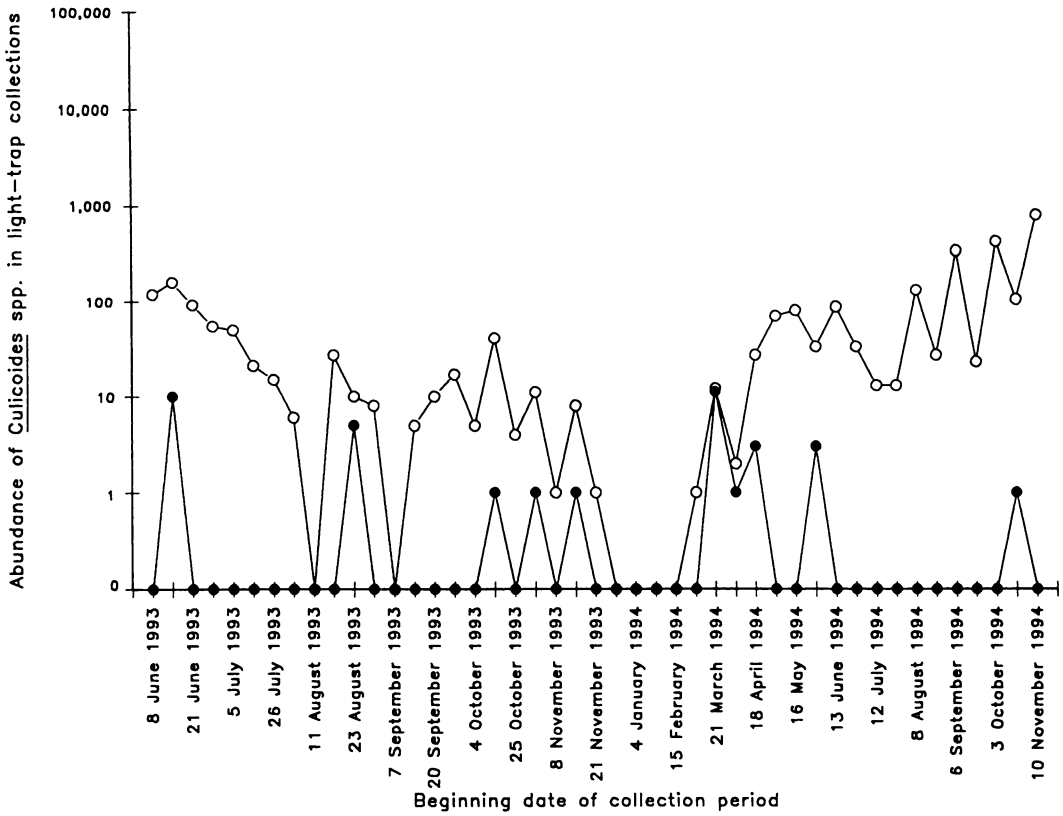

FIGURE 7. Abundance of *C. venustus* (○) and *C. variipennis* (●) in light trap collections, by collection period. See Fig. 3 for explanation.

that *C. lahillei* can become infected with BT virus serotype 11 after feeding on an infected blood suspension, albeit at a low prevalence (1.9%) (Mullen et al., 1985b). Based on seasonality and relative abundance in deer and light-trap collections, *Culicoides stellifer* and *C. spinosus* also were considered suspect vectors at CWMA.

Less likely suspect vectors included *C. paraensis, C. venustus, C. biguttatus,* and *C. niger. Culicoides paraensis,* while collected from deer only in low numbers, had a distinct summer and fall seasonality. *Culicoides venustus* was consistently present in light-trap collections, and Schmidtmann et al. (1980) demonstrated it to be primarily nocturnal in activity. Therefore, *C. venustus* may have attacked deer at CWMA more commonly than was indicated by crepuscular collections from the captive deer. Laboratory studies with *C. venustus* from New York resulted in prevalence of infection of 2.6% with an EHD virus and 0.7% with BT virus serotypes 10 and 11 (Jones et al., 1983).

Although the spring seasonality of *C. biguttatus* and *C. niger* does not coincide with the late summer and early fall seasonality of clinical HD, a possible role of these species in early season transmission of EHD and BT viruses among white-tailed deer should not be ruled out. In a 1981 Florida study, Greiner et al. (1985) reported isolates of EHDV-2 from seven pools of parous *C. variipennis* associated with cattle during April (n = 1) and May (n = 1) as well as June (n = 4) and July (n = 1).

Based on the extremely low numbers of *C. variipennis, C. insignis, C. obsoletus/sanguisuga,* and other *Culicoides* spp.

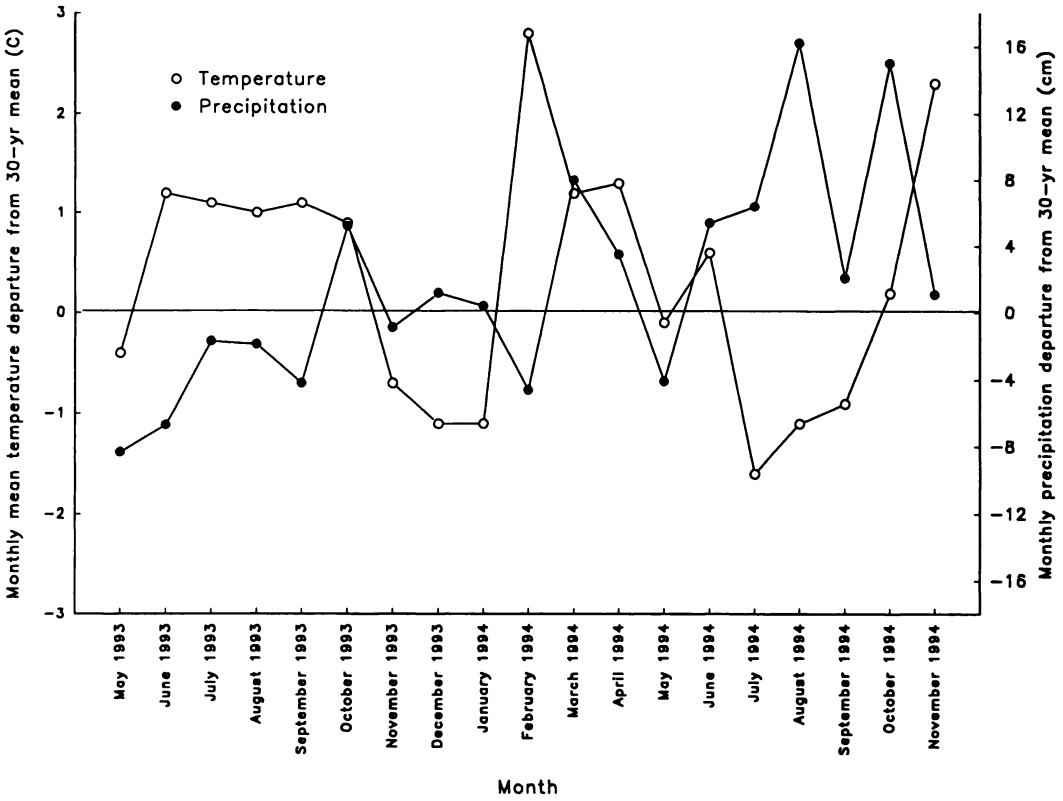

FIGURE 8. Monthly mean temperature and monthly precipitation departures from 30-yr means for area of study site.

in CWMA collections, these taxa were not considered to have been likely vectors of EHD and BT viruses for deer at CWMA in our study. The absence of *C. variipennis* in deer collections at CWMA was consistent with its absence in collections from white-tailed deer in Alabama (USA) (Mullen et al., 1985a) and from white-tailed deer-baited drop traps in Tennessee (USA) (Gerhardt, 1986). Significant host-seeking activity by *C. variipennis* during crepuscular periods, particularly during the evening, has been documented (Zimmerman and Turner, 1983; Mullens, 1995). Thus, collections from deer during crepuscular periods should yield *C. variipennis* if this species commonly attacks deer at a given trapping locale. The rarity of *C. variipennis* in light-trap collections at CWMA was consistent with results from other trapping efforts in natural woodland settings in the southeastern United States (Tanner and Turner, 1975; Kline and Greiner, 1992; K. E. Smith, unpubl.) and was additional evidence that *C. variipennis* was very uncommon at CWMA. The presence of *C. insignis* at CWMA extends the reported range of this species beyond Florida and coastal Georgia (Blanton and Wirth, 1979; Hagan and Wirth, 1985).

The *Culicoides* spp. collected from deer at CWMA were identical to those collected from white-tailed deer during previous studies in Alabama (Mullen et al., 1985a) and Tennessee (Gerhardt, 1986), with three minor exceptions. *Culicoides haematopotus* and *C. arboricola* were collected from deer at CWMA (albeit rarely), but not in Alabama or Tennessee. *Culicoides bickleyi* was present only in the Tennessee collections. The Alabama study also was similar to the Georgia study reported here in that, based on seasonality and relative abundance, *C. lahillei* and *C. stellifer* rep-

resent the two most likely vectors of EHD and BT viruses for white-tailed deer in the southeastern United States (Mullen et al., 1985a). The Tennessee study differed in that *C. paraensis* and *C. obsoletus/sanguisuga* were the predominant species collected during the summer and fall months (Gerhardt, 1986). However, the Tennessee collections were made only during evening crepuscular periods, which probably resulted in an underestimation of the abundance of *C. lahillei* and possibly *C. stellifer*.

Several *Culicoides* spp. were more abundant during the summer and fall of 1994 than during the corresponding period of 1993. The relatively low numbers of *Culicoides* spp. in 1993 collections probably was due to the hot, dry weather experienced during June through September. The cooler, wetter conditions during the summer of 1994 provided more favorable conditions for populations of at least some *Culicoides* spp., and thus may have been an important factor in the increased virus activity observed during that year.

The lack of virus isolations during 1993 is not surprising given the very low level of virus activity at CWMA during that year. The lack of virus isolations during 1994, when virus activity was higher, is problematic but does not rule out transmission by *Culicoides* spp. In previous studies in enzootic areas such as Florida, Louisiana (USA), the Caribbean, and Central America, BT and EHD virus isolation or positive polymerase chain reaction rates have been reported from confirmed *Culicoides* sp. vectors ranging from zero to one positive per 1,000 to 2,000 parous females tested (Greiner et al., 1985, 1989, 1993; Wieser-Schimpf et el., 1993; Mo et al., 1994). These low isolation prevalences despite collection of midges near concentrations of domestic ruminant hosts illustrate the difficulty of obtaining isolates from midges in enzootic areas.

A partial explanation for our lack of virus isolations is that most females tested probably were nulliparous. Nulliparous females were included in virus isolation attempts for two reasons. First, simple visual techniques to distinguish parous females of most of the species collected have not been confirmed and published. Second, the possibility of transovarial transmission of EHD or BT viruses cannot be ruled out. Transovarial transmission trials with BT viruses so far have been negative, but no such work has been done with EHD viruses. If transovarial transmission of these viruses does not occur, nulliparous females would never be infected. Thus, it is possible that the effective sample size of *Culicoides* spp. was much smaller than reported. Furthermore, it is possible that potential vector species of *Culicoides* spp. were infected with viruses at a low prevalence, as has been observed in experimental infections of *C. lahillei* with EHD virus (Smith et al., 1996).

Our virus isolation protocol may not have been as sensitive as needed to pick up low prevalence infections. Egg inoculation is more sensitive for BT viruses than is cell culture (Wechsler and McHolland, 1988), and in a previous study (Gibbs and Greiner, 1983) a mosquito cell line was more sensitive for isolating EHD viruses from midges than were BHK_{21} cells.

ACKNOWLEDGMENTS

This research was conducted through sponsorship from the fish and wildlife agencies of Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia. Funds were administered and coordinated under the Federal Aid to Wildlife Restoration Act (50 Stat. 917). Support also was received through Grant Agreements 1448-0004-93-909, Fish and Wildlife Service, and 14-45-0009-94-906, National Biological Service, U.S. Department of the Interior, and through Cooperative Agreement 94-9-6-13-0032-CA, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture. Additional support was provided through grant number 29-26-GR207-002 from the Veterinary Medical Experiment Station, College of Veterinary Medicine, The University of Georgia. The authors thank St. Joseph's Paper Company and the GDNR for allowing use of CWMA and facilities on the area. The time and efforts of

GDNR personnel, particularly Ronald Simpson, Mike Rowell, Ralph Rumph, and Gerald Henry, are greatly appreciated. We also thank Fred Holbrook and Greg Hunt of the United States Department of Agriculture, Agricultural Research Service, Arthropod-borne Animal Diseases Research Laboratory for providing EHD virus-infected *Culicoides variipennis*. Thanks go to Victor Nettles and Randy Davidson for guidance throughout the project. Finally, Southeastern Cooperative Wildlife Disease staff members, particularly Lenus Hall, Darrell Kavanaugh, Kevin Keel, Lauren Richey, and Jennifer Smith deserve special recognition for their technical assistance.

LITERATURE CITED

BLANTON, F. S., AND W. W. WIRTH. 1979. Arthropods of Florida and neighboring land areas, Vol. 10: The sand flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae). Florida Department of Agriculture and Consumer Services, Gainesville, Florida, 204 pp.

COUVILLION, C. E., V. F. NETTLES, W. R. DAVIDSON, J. E. PEARSON, AND G. A. GUSTAFSON. 1981. Hemorrhagic disease among white-tailed deer in the Southeast from 1971–1980. Proceedings of the United States Animal Health Association 85: 522–537.

DEAN, A. G., J. A. DEAN, D. COULOMBIER, K. A. BRENDEL, D. C. SMITH, A. H. BURTON, R. C. DICKER, K. SULLIVAN, R. F. FAGAN, AND T. G. ARNER. 1994. Epi Info, Version 6: A word processing, database, and statistics program for epidemiology on microcomputers. Centers for Disease Control and Prevention, Atlanta, Georgia, 601 pp.

EVE, J. H., AND F. E. KELLOGG. 1977. Management implications of abomasal parasites in southeastern white-tailed deer. The Journal of Wildlife Management 41: 169–177.

FOSTER, N. M., R. D. BRECKON, A. J. LUEDKE, R. H. JONES, AND H. E. METCALF. 1977. Transmission of two strains of epizootic hemorrhagic disease virus in deer by *Culicoides variipennis*. Journal of Wildlife Diseases 13: 9–16.

GERHARDT, R. R. 1986. *Culicoides* spp. attracted to ruminants in the Great Smoky Mountains National Park, Tennessee. Journal of Agricultural Entomology 3: 192–197.

GIBBS, E. P. J., AND E. C. GREINER. 1983. Bluetongue infections and *Culicoides* species associated with livestock in Florida and the Caribbean region. *In* Double-stranded RNA viruses, R. C. Compans and D. H. L. Bishop (eds.). Elsevier Science Publishing Co., New York, New York, pp. 375–382.

———, AND ———. 1989. Bluetongue and epizootic hemorrhagic disease. *In* Arboviruses: Epidemiology and ecology, Vol 2. T. Monath (ed.). CRC Press, Boca Raton, Florida, pp. 39–70.

GREINER, E. C., T. L. BARBER, J. E. PEARSON, W. L. KRAMER, AND E. P. J. GIBBS. 1985. Orbiviruses from *Culicoides* in Florida. Progress in Clinical and Biological Research 178: 195–200.

———, F. C. M. ALEXANDER, J. ROACH, V. MOE, G. BORDE, W. P. TAYLOR, J. DICKINSON, AND E. P. J. GIBBS. 1989. Bluetongue epidemiology in the Caribbean region: Serological and entomological findings from a pilot sentinel system in Trinidad and Tobago. Medical and Veterinary Entomology 3: 101–105.

———, C. L. MOE, E. J. HOMAN, J. GONZALEZ, M. T. OVIEDO, L. H. THOMPSON, AND E. P. J. GIBBS. 1993. Epidemiology of bluetongue in Central America and the Caribbean: Initial entomological findings. Medical and Veterinary Entomology 7: 309–315.

HAGAN, D. V., AND W. W. WIRTH. 1985. New distribution records of *Culicoides* species from coastal Georgia. Journal of Agricultural Entomology 2: 207–211.

HAYES, M. E., G. R. MULLEN, AND K. E. NUSBAUM. 1984. Comparison of *Culicoides* spp. (Diptera: Ceratopogonidae) attracted to cattle in an open pasture and bordering woodland. Mosquito News 44: 368–370.

JONES, R. H. 1985. Vector research with the orbiviruses. Progress in Clinical and Biological Research 178: 147–149.

———, R. D. ROUGHTON, N. M. FOSTER, AND B. M. BANDO. 1977. *Culicoides*, the vector of epizootic hemorrhagic disease in white-tailed deer in Kentucky in 1971. Journal of Wildlife Diseases 13: 2–8.

———, E. T. SCHMIDTMANN, AND N. M. FOSTER. 1983. Vector-competence studies for bluetongue and epizootic hemorrhagic disease viruses with *Culicoides venustus*. Mosquito News 43: 184–186.

KLINE, D. L., AND E. C. GREINER. 1992. Field observations on the ecology of adult and immature stages of *Culicoides* spp. associated with livestock in Florida, USA. *In* Bluetongue, African horse sickness and related orbiviruses, T. E. Walton and B. I. Osburn (eds.). CRC Press, Boca Raton, Florida, pp. 896–905.

MO, C. L., L. H. THOMPSON, E. J. HOMAN, M. T. OVIEDO, E. C. GREINER, J. GONZALEZ, AND M. R. SAENZ. 1994. Bluetongue virus isolations from vectors and ruminants in Central America and the Caribbean. American Journal of Veterinary Research 55: 211–215.

MULLEN, G. R., M. E. HAYES, AND K. E. NUSBAUM. 1985a. Potential vectors of bluetongue and epizootic hemorrhagic disease viruses of cattle and white-tailed deer in Alabama. Progress in Clinical and Biological Research 178: 201–206.

———, R. H. JONES, Y. BRAVERMAN, AND K. E.

NUSBAUM. 1985b. Laboratory infections of *Culicoides debilipalpis* and *C. stellifer* (Diptera: Ceratopogonidae) with bluetongue virus. Progress in Clinical and Biological Research 178: 239–243.

MULLENS, B. A. 1992. Integrated management of *Culicoides variipennis:* A problem of applied ecology. *In* Bluetongue, African horse sickness and related orbiviruses, T. E. Walton and B. I. Osburn (eds.). CRC Press, Boca Raton, Florida, pp. 896–905.

———. 1995. Flight activity and response to carbon dioxide of *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) in southern California. Journal of Medical Entomology 32: 310–315.

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION. 1993. Climatological data, Georgia, May–December 1993. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Climatic Data Center, Asheville, North Carolina. Vol. 97, numbers 5–12.

———. 1994. Climatological data, Georgia, January–November 1994. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Climatic Data Center, Asheville, North Carolina. Vol. 98, numbers 1–11.

NETTLES, V. F., AND D. E. STALLKNECHT. 1992. History and progress in the study of hemorrhagic disease of deer. Transactions of the North American Wildlife and Natural Resources Conference 57: 499–516.

———, W. R. DAVIDSON AND D. E. STALLKNECHT. 1992. Surveillance for hemorrhagic disease in white-tailed deer and other wild ruminants, 1980–1989. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 46: 138–146.

NEVILL, E. M., B. J. ERASMUS, AND G. J. VENTER. 1992. A six-year survey of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae). *In* Bluetongue, African horse sickness and related orbiviruses, T. E. Walton and B. I. Osburn (eds.). CRC Press, Boca Raton, Florida, pp. 314–319.

PEARSON, J. E., AND M. M. JOCHIM. 1979. Protocol for the immunodiffusion test for bluetongue. Proceedings of the American Association of Veterinary Laboratory Diagnosticians 22: 463–475.

PRESTWOOD, A. K., T. P. KISTNER, F. E. KELLOGG, AND F. A. HAYES. 1974. The 1971 outbreak of hemorrhagic disease among white-tailed deer of the southeastern United States. Journal of Wildlife Diseases 10: 217–224.

SCHMIDTMANN, E. T., J. F. ABEND, AND M. E. VALLA. 1980. Nocturnal blood-feeding from pastured calves by the ceratopogonid midge, *Culicoides venustus,* in New York State. Mosquito News 40: 571–577.

SEVERINGHAUS, C. W. 1949. Tooth development and wear as a criteria of age in white-tailed deer of the southeastern United States. The Journal of Wildlife Management 13: 195–216.

SMITH, K. E., AND D. E. STALLKNECHT. 1996. *Culicoides* (Diptera: Ceratopogonidae) collected during epizootics of hemorrhagic disease among captive white-tailed deer. Journal of Medical Entomology 33: in press.

———, ———, AND V. F. NETTLES. 1996. Experimental infection of *Culicoides lahillei* (Diptera: Ceratopogonidae) with epizootic hemorrhagic disease virus serotype 2 (Orbivirus: Reoviridae). Journal of Medical Entomology 33: 117–122.

STALLKNECHT, D. E., J. L. BLUE, E. A. ROLLER, V. F. NETTLES, W. R. DAVIDSON, AND J. E. PEARSON. 1991. Precipitating antibodies to epizootic hemorrhagic disease and bluetongue viruses in white-tailed deer in the southeastern United States. Journal of Wildlife Disease 27: 238–247.

———, V. F. NETTLES, E. A. ROLLOR, III, AND E. W. HOWERTH. 1995. Epizootic hemorrhagic disease virus and bluetongue virus serotype distribution in white-tailed deer in Georgia. Journal of Wildlife Diseases 31: 331–338.

TANNER, G. D., AND E. C. TURNER, JR. 1975. Seasonal abundance of *Culicoides* spp. as determined by three trapping methods. Journal of Medical Entomology 12: 87–91.

TANYA, V. N., E. C. GREINER, AND E. P. J. GIBBS. 1992. Evaluation of *Culicoides insignis* (Diptera: Ceratopogonidae) as a vector of bluetongue virus. Veterinary Microbiology 32: 1–14.

THOMAS, F. C. 1981. Hemorrhagic disease. *In* Diseases and parasites of white-tailed deer, W. R. Davidson, F. A. Hayes, V. F. Nettles, and F. E. Kellogg (eds.). Miscellaneous Publication No. 7, Tall Timbers Research Station, Tallahassee, Florida, pp. 87–96.

WECHSLER, S. J. AND L. E. MCHOLLAND. 1988. Susceptibilities of 14 cell lines to bluetongue virus infection. Journal of Clinical Microbiology 26: 2324–2327.

WIESER-SCHIMPF, L., W. C. WILSON, D. D. FRENCH, A. BAHAM, AND L. D. FOIL. 1993. Bluetongue virus in sheep and cattle and *Culicoides variipennis* and *C. stellifer* (Diptera: Ceratopogonidae) in Louisiana. Journal of Medical Entomology 30: 719–724.

ZIMMERMAN, R. H., AND E. C. TURNER, JR. 1983. Host-feeding patterns of *Culicoides* (Diptera: Ceratopogonidae) collected from livestock in Virginia, USA. Journal of Medical Entomology 20: 514–519.

Received for publication 2 October 1995.