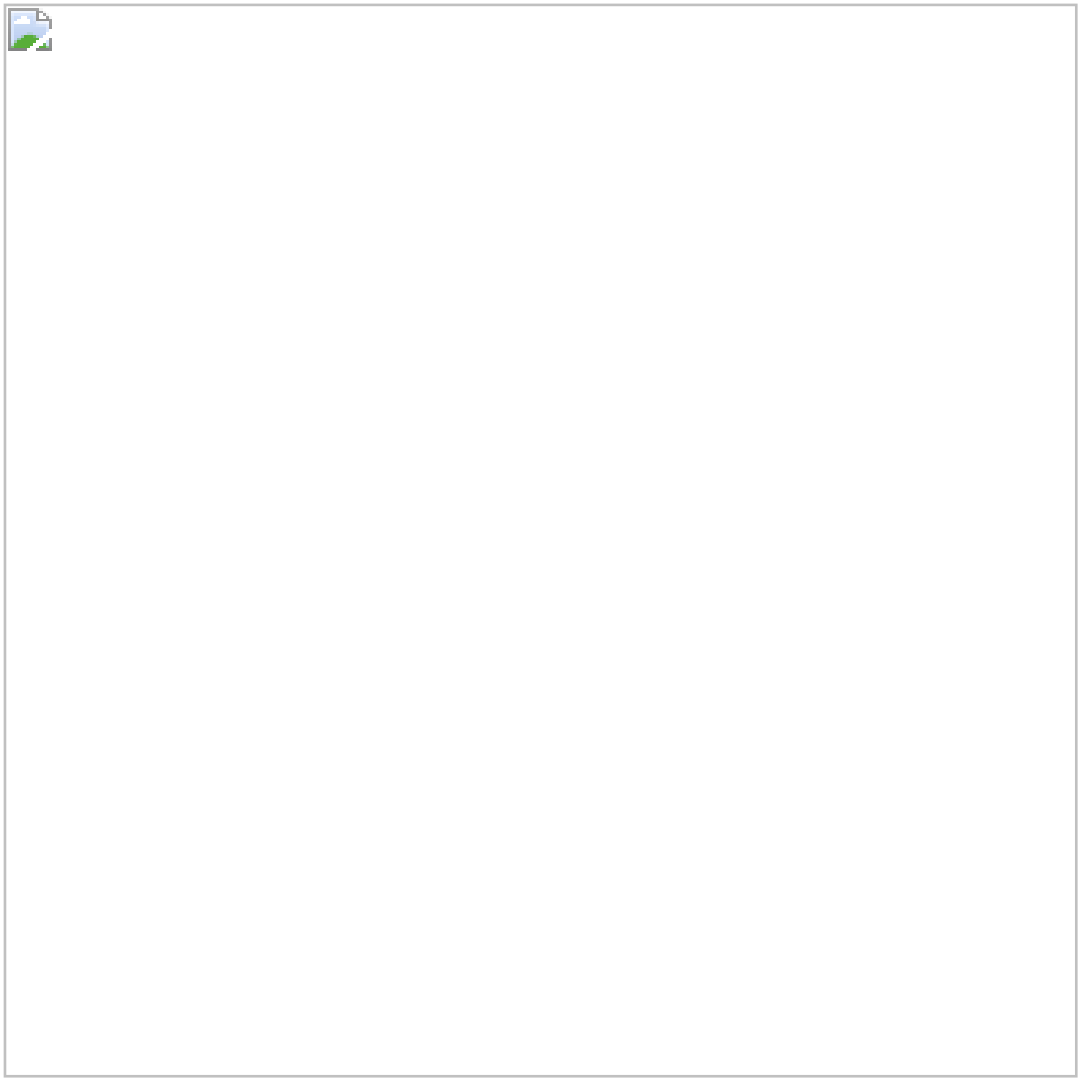

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Biting midges of the genus *Culicoides* in South Carolina zoos

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Abstract

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) were collected during the summer of 2007 at the Greenville and Riverbanks Zoos in South Carolina with Centers for Disease Control and Prevention (CDC) traps equipped with ultraviolet or incandescent lights and baited with carbon dioxide. Sixteen species of *Culicoides* were collected, four of which represented more than 80%. They were *Culicoides guttipennis* (Coquillett), *Culicoides mulrenanni* Beck, *Culicoides obsoletus* (Meigen), and *Culicoides sanguisuga* (Coquillett). *C. guttipennis* was found on a dead colobus monkey and a dead golden-headed lion tamarin; *Culicoides husseyi* Wirth & Blanton was collected from an unidentified, abandoned bird's nest. Ultraviolet light-equipped traps captured significantly more *Culicoides* specimens than traps with incandescent light. Half of the collected species previously have been associated with vertebrate pathogens, indicating a potential risk to captive animals.

Keywords: biting flies, blood feeding, exotic animals, light traps, vectors
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Introduction

Zoos can provide a valuable surveillance system for a variety of zoonoses. The initial isolation and identification of West Nile virus in the United States, for example, was performed by employees of the Bronx Zoo, New York, NY, in 1999 (Centers for Disease Control and Prevention 1999). This initial isolation and recognition of West Nile virus became the impetus for a mosquito-surveillance program in the Bronx Zoo. However, most other American zoos have not initiated similar surveys to detect potential epizootics (Nelder, unpublished). Eastern equine encephalitis virus was detected in whooping cranes, *Grus americana*, from the Patuxent Wildlife Research Center, Laurel, Maryland (Dein et al. 1986). The etiological agent of tularemia, *Francisella tularensis*, was detected in a squirrel monkey, *Saimiri* sp. (ca. 1989) and another unidentified monkey species (ca. 1988) in the Sacramento Zoo, California (Farlow et al. 2001). Epizootics in zoos, therefore, offer excellent opportunities to study the epidemiology of diseases with the ultimate goal of preventing further epizootics and epidemics.

While the presence of arthropods such as mosquitoes and cockroaches has been documented in zoological settings (Greenberg and Sanati 1970), biting midges have been overlooked, though there have been reports of the pathogens transmitted by these flies. For example, viral pathogens of birds (*Avipoxvirus* spp.), transmitted by biting midges and other biting flies, have been reported from two species of captive owls in two facilities in Florida (Deem et al. 1997). Species of *Haemoproteus*, avian blood parasites transmitted by biting midges and other biting flies, have been recorded from infected birds in zoos of California (Schrenzel et al. 2003; Ziman et al. 2004), Michigan (DeJong and Muzzall 2000), Oklahoma (Halpern and Bennett 1983), and Texas (Ferrell et al. 2007). These cases demonstrate that zoos can be foci of ceratopogonid-borne zoonoses, indicating the need for collaboration between veterinarians and entomologists.

To protect endangered animals and those in conservation and breeding programs, potential threats posed by biting midges should be evaluated. The objectives of this research were to survey biting midges and assess CDC traps equipped with ultraviolet or incandescent lights for their capture efficiency in two zoos in South Carolina: the Greenville Zoo in Greenville and the Riverbanks Zoo in Columbia.

Materials and Methods

Study sites

The Greenville Zoo (N 34° 50.58′ W 82° 23.24′; ~294 MASL) is situated in the Western Division of the Piedmont on 4.5 hectares bordered by the Reedy River (5-10 m wide), a 1 m-wide unnamed stream, businesses, and residential areas. The Riverbanks Zoo and Botanical Gardens (N 34° 00.58′ W 81° 04.56′; ~51 MASL) occupies 69 hectares in Columbia near the confluence of the Saluda and Broad Rivers. It is in the Sandhills Ecoregion on the Fall Line of the extreme western edge of the Atlantic Coastal Plain and is bordered by Interstate 126, business properties, and recreational areas.

Collection of biting midges

Biting midges (*Culicoides* spp. (Diptera: Ceratopogonidae)) were collected with two

types of traps: CDC traps with incandescent light (4-Watt, ABC trap, Clarke Mosquito Control, www.clarkemosquito.com) and CDC traps with ultraviolet light (Trap Model 1212, John W. Hock Company, www.johnwhock.com). All traps were suspended from trees at 1.5-2.0 m above ground in forests with approximately 50% canopy cover dominated by oaks, pines, and bamboo *Phyllostachys aurea* Carr. ex A. & C. Rivière (Cyperales: Poaceae). Ground cover consisted of woody plants such as poison ivy *Toxicodendron radicans* L. (Sapindales: Anacardiaceae) and Virginia creeper *Parthenocissus quinquefolia* L. (Vitales: Fitaceae). To provide a source of CO_2, all traps were baited with 2.0-2.5 kg of dry ice in insulated dry-ice containers placed immediately above the traps (Igloo, Trap Model 812, John W. Hock Company). All traps were powered by 6-V, 12-amps per hour, rechargeable batteries (Model PS-6100F1, Power Sonic®, www.power-sonic.com).

Biting midges were collected from May to August 2007, using incandescent and UV traps. Two traps of each type were used in the Greenville Zoo, and three of each type were used in the Riverbanks Zoo. Incandescent and UV traps were run simultaneously for four consecutive nights per month, beginning at 16:00 h, and trap-collection containers (containing live biting midges) were retrieved at 08:00 h each of the four consecutive mornings. A trap night was defined as the period from deployment of a single trap until collection the next morning. Dead animals and nests, when available, also were examined for flies.

All specimens were fixed in 95% ethanol for subsequent clearing in phenol-alcohol and slide mounted in phenol-balsam, following the methods of Wirth and Marston (1968). *Culicoides* species were identified with keys and illustrations by Battle and Turner (1971), Blanton and Wirth (1979), and Wirth et al. (1985) and by comparison with specimens in the collection of WL Grogan. Representative specimens were deposited in the Clemson University Arthropod Collection and the synoptic collection of ceratopogonids maintained by WLG at Salisbury University.

Statistical analyses

The mean numbers of biting midges per trap night were analyzed using a two-way ANOVA, comparing means between trap types, using month as a block, and following the general statistical methodology of Zar (1996). Tests were considered significant at $p < 0.05$.

Results

Sixteen species of *Culicoides* (Diptera: Ceratopogonidae) were collected from the zoos, with 10 species from the Greenville Zoo and 12 species from the Riverbanks Zoo (Table 1). Six species were present at both zoos: *Culicoides guttipennis* (Coquillett), *C. obsoletus* (Meigen), *C. paraensis* (Goeldi), *C. sanguisuga* (Coquillett), *C. scanloni* Wirth and Hubert, and *C. stellifer* (Coquillett). A total of 101 biting midges were collected at the Greenville Zoo, 90% of which were represented by two species, *C. guttipennis* (*n* = 59) and *C. obsoletus* (*n* = 32). At the Riverbanks Zoo, 88 specimens of *Culicoides* were captured, 81% of which belonged to four species: *C. sanguisuga* (*n* = 31), *C. mulrenanni* Beck (*n* = 25), *C. stellifer* (*n* = 8), and *C. guttipennis* (*n* = 7).

The incandescent traps, for both zoos combined, collected 38 individuals, representing 20% of the total number of *Culicoides* trapped. In the Greenville Zoo, the

mean number of *C. guttipennis* per trap night was significantly higher in UV traps than in incandescent traps ($F_{2, 45}$ = 12.8, p = 0.001) (Table 2). In the Riverbanks Zoo, the mean number of *C. mulrenanni* per trap night was not significantly higher in UV traps than in incandescent traps ($F_{2, 45}$ = 3.7, p = 0.06). Mean numbers of *C. obsoletus* and *C. sanguisuga* caught per trap night were not analyzed statistically because they were not collected in incandescent traps.

Culicoides species were not observed feeding in the zoo, but females of *C. guttipennis* were collected from a dead colobus monkey, *Colobus guereza* Rüppell (Haplorrhini: Cercopithecidae), (one of two animals examined) and a dead golden-headed lion tamarin, *Leontopithecus chrysomelas* (Kuhl) (Haplorrhini: Cebidae), (one of three animals examined) in the Greenville Zoo. *C. husseyi* Wirth & Blanton was collected from one of four unidentified, abandoned bird nests in the Greenville Zoo.

Discussion

The 16 species of *Culicoides* collected in both zoos were typical of those in more rural areas of South Carolina. Suitable habitats for the immature stages of *Culicoides* are often abundant in zoos. The relatively higher numbers of *C. obsoletus* and *C. sanguisuga* in this study are likely due to the abundance of larval habitats of these two species in the zoos, including moist leaves and dung-laden straw (Jamnback 1965). *C. guttipennis* inhabits water-filled treeholes, a habitat found throughout the zoos and surrounding properties.

The collection of the coastal, salt marsh species, *Culicoides furens* (Poey), with a UV trap in the Riverbanks Zoo was unexpected. Adults of this species in the US Virgin Islands might be carried by prevailing winds for at least 6.4 km over low mountains (366 m) (Williams 1962), and adults in Panama could be carried up to 3.2 km by winds (Breeland and Smith 1962).

Table 1. Biting midges collected from the Greenville Zoo (GVZ) and the Riverbanks Zoo (RBZ), South Carolina, with notes on the traps used to collect each species.

Species	Numbers per zoo		
	GVZ	RBZ	Trap[1]
Culicoides biguttatus Coquillett	0	4	CDC-INC, CDC-UV
Culicoides crepuscularis Malloch	0	1	CDC-UV
Culicoides debilipalpis (Lutz)	1	0	CDC-UV
Culicoides furens (Poey)	0	1	CDC-UV
Culicoides guttipennis (Coquillett) [2]	59	7	CDC-INC, CDC-UV
Culicoides haematopotus Malloch	0	3	CDC-INC, CDC-UV
Culicoides hinmani Khalaf	0	1	CDC-UV
Culicoides husseyi Wirth and Blanton[3]	1	0	NA
Culicoides mulrennani Beck	0	24	CDC-INC, CDC-UV
Culicoides obsoletus (Meigen)	32	3	CDC-UV
Culicoides ousairani Khalaf	1	0	CDC-UV
Culicoides paraensis (Goeldi)	2	3	CDC-UV
Culicoides sanguisuga (Coquillett)	4	31	CDC-UV
Culicoides scanloni Wirth and Hubert	1	2	CDC-UV
Culicoides stellifer(Coquillett)	1	8	CDC-INC, CDC-UV
Culicoides villosipennis Root & Hoffman	1	0	CDC-UV

[1] Centers for Disease Control (CDC) traps with incandescent light (CDC-INC) and CDC traps with ultraviolet light (CDC-UV).
[2] ex. golden-headed lion tamarin (GVZ, *n* = 1), ex. colobus monkey (GVZ, *n* = 1)
[3] ex. unidentified bird nest (GVZ, *n* = 1).

To our knowledge, *C. furens* has never been collected as far inland as central South Carolina; it possibly reached this locale by following the Congaree River inland from coastal habitats. The salt marsh mosquito *Ochlerotatus taeniorhynchus* Wiedemann (Diptera: Culicidae) is routinely collected in the vicinity of the Riverbanks Zoo (Nelder, unpublished data).

Several species of *Culicoides* in this study have been associated with vertebrate pathogens (Table 3). For example, *C. stellifer* harbors Bluetongue virus in Alabama (Mullen and Anderson 1998) and West Nile virus in Louisiana (Sabio et al. 2006). Several species of *Culicoides* (e.g., *C. crepuscularis, C. haematopotus*, and *C. hinmani*) are vectors of *Haemoproteus* spp. and filarial parasites. Some species also might transmit nematodes, protozoans, and viruses to captive animals in zoos. *C. obsoletus* is a major vector of Bluetongue virus-8 in Europe and is the cause of epidemics throughout northern Europe (Mehlhorn et al. 2007). However, in the United States and Canada, *C. sonorensis* Wirth & Jones is the primary vector of Bluetongue virus (Borkent 2005), and *C. obsoletus* does not appear to be an effective vector in the Nearctic Region. Several other pathogens, if introduced into the United States, could possibly be transmitted by native species of *Culicoides*. For example, *C. paraensis* was collected from both zoos and is the only known vector of Oropouche virus, which causes nonfatal, febrile, flu-like infections in humans in South America and has been isolated from other primates and sloths (Mullen 2002).

In zoos of South Carolina, surveillance for biting midges would best be served using UV traps because of the relatively greater abundance of biting midges caught by these traps and because no species was collected exclusively in incandescent traps. Competition from other incandescent light sources in and around a zoo likely reduces the collection efficiency of incandescent traps. The efficiency of traps for capturing biting midges differed geographically, and surveillance measures might need to be tailored for specific zoos. Surveillance programs should take into account the height at which biting midges seek hosts in the canopy. Some species in our study, such as *C. crepuscularis* Malloch, *C. haematopotus* Malloch, *C. hinmani* Khalaf, *C. scanloni*, and *C. villosipennis* Root & Hoffman, more frequently are collected higher in the canopy (Swanson 2007). Surveillance programs at zoos should consider the target species and vary the height of traps to sample the regional biting midge community.

Table 2. Mean number of *Culicoides* per trap night for the two most commonly collected species at the Greenville and Riverbanks Zoos, South Carolina, for two CDC-trap types, May-August 2007.

Zoo and *Culicoides* species	***Culicoides* per trap night (Mean ± SE)[1]**	
	CDC-UV[2]	**CDC-INC[2]**
Greenville Zoo		
C. guttipennis	1.8 ± 0.73a	0.65 ± 0.30b
C. obsoletus	1.3 ± 0.99	0.0 ± 0.00
Riverbanks Zoo		
C. mulrennani	0.67 ± 0.42a	0.29 ± 0.17a
C. sanguisuga	1.2 ± 0.49	0.0 ± 0.00

[1] Means followed by different letters, within species at each zoo, are significantly different at $p < 0.05$.
[2] Centers for Disease Control (CDC) traps with incandescent light (CDC-INC) and CDC traps with ultraviolet light (CDC-UV).

Table 3. Larval habitats, host-feeding records, and pathogens associated with biting midges (*Culicoides*) collected at the Greenville and Riverbanks Zoos, South Carolina.

Species	Larval habitats	Host(s)	Vertebrate pathogens or conditions (reference)
C. biguttatus	Mud, decaying organic matter	Mammals and birds	West Nile virus (Sabio et al. 2006)
C. crepuscularis	Moist terrestrial habitats, edges of streams and ponds	Birds	Bluetongue virus (White et al. 2005)
			Chandlerrella quiscali (Robinson 1971)
			Eufilaria longicaudata (Hibler 1963)
			Haemoproteus spp. (Fallis and Bennett 1961)
			Splendidofilaria procardina (Hibler 1963)
			Trypanosoma spp. (Bennett 1961)
C. debilipalpis	Tree holes, decaying organic matter	Mammals and birds	None reported
C. furens	Salt marshes and streams	Mammals, some birds	*Mansonella ozzardi* (Lowrie and Raccurt 1981)
			Tetrapetalonema marmosetae (Lowrie et al. 1978)
C. guttipennis	Tree holes	Mammals	None reported
C. haematopotus	Moist terrestrial habitats, edges of streams and ponds	Birds, some mammals	*Chandlerrella quiscala* (Robinson 1971)
			Chandlerrella striatospicula (Hibler 1963)
			Eufilaria longicaudata (Hibler 1963)
			Haemoproteus meleagridis (Atkinson 1988)
C. hinmani	Tree holes	Mammals	*Haemoproteus meleagridis* (Atkinson 1988)
C. husseyi	Unknown	Unknown	None reported
C. mulrennani	Moist terrestrial habitats, edges of streams and ponds	Mammals, some birds	None reported
C. obsoletus	Mud, straw mixed with dung, moist leaves	Mammals	Allergic dermatitis in horses (Kleider and Lees 1984)
			Bluetongue virus (Mehlhorn et al. 2007)
C. ousairani	Tree holes	Birds	None reported
C. paraensis	Tree holes	Mammals and birds	*Mansonella ozzardi*
			Oropouche virus (Pinheiro et al. 1981)
C. sanguisuga	Mud, straw mixed with dung, moist leaves	Mammals	None reported
C. scanloni	Moist terrestrial habitats, edges of streams and ponds	Birds	None reported
C. stellifer	Moist terrestrial habitats, edges of streams and ponds	Mammals	Allergic dermatitis in horses (Greiner et al. 1988)
			Bluetongue virus (Mullen and Anderson 1998)
			Vesicular stomatitis virus (Walton et al. 1987)
			West Nile virus (Sabio et al. 2006)
C. villosipennis	Tree holes	Birds, some mammals	None reported

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