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Characterizing the epidemiology of bluetongue virus serotype one in south Louisiana

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CHARACTERIZING THE EPIDEMIOLOGY OF BLUETONGUE VIRUS SEROTYPE ONE IN SOUTH LOUISIANA

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ABSTRACT

In November 2004, bluetongue virus (BTV) serotype 1 was detected for the first time in the U.S. from a hunter-killed deer in the marsh area of the Atchafalaya Delta in St. Mary Parish, LA. Subsequent serum surveys of three cattle farms further inland from the area where the deer was shot found bluetongue virus serotype 1 positive cattle on two of the three farms. The purpose of this study was to determine potential BTV vectors in the area where BTV-1 positive animals were detected and compare different trapping techniques for capturing specimens of the genus *Culicoides*. The study was conducted from January 2006 through November 2007. Seven sites were established in the immediate area (marsh) where the deer was found and miniature CDC light traps were deployed once per month at each site. At each of the three cattle farms, two CDC light traps (one with and one without dry ice) were deployed twice per month. In 2007, New Jersey traps with incandescent bulbs or black light bulbs were compared to CDC traps baited with dry ice. Specimens of 10 different species of *Culicoides* were captured at the farms and specimens of 7 of these 10 species were caught in the Atchafalaya Delta marsh area. In the entire study, 8,179 ceratopogonids were captured including 5,068 of the genus *Culicoides*. CDC light traps baited with dry ice caught significantly more flies than traps without dry ice. Infrared reverse transcriptase polymerase chain reaction was performed to screen for BTV in 275 pools representing 2,504 specimens collected at the farms. All positive samples were sequenced for serotype determination. Five pools out of 275 (1.8%) were positive for BTV. Pools of four species of *Culicoides* were found to be positive: *C. crepuscularis, C. debilipalpis* (2 pools), *C. haematopotus,* and *C. furens*. The amplicons of the positive specimens were sequenced and found to be identical to

either BTV-17 or BTV-13. Since we did not detect BTV-1 in any biting midges, future studies will be necessary to establish the epidemiology of bluetongue virus serotype 1 in south Louisiana.

INTRODUCTION

Bluetongue virus (BTV), an *Orbivirus* in the family Reoviridae, is a double-stranded, segmented RNA virus known to infect many different domestic and wild ruminants. Bluetongue disease is on the multispecies list of notifiable diseases by the Office of International Epizootics because of its substantial economic impact and potential for rapid spread. Infection with BTV can cause serious hemorrhagic disease with high mortality rates in sheep and deer (Osburn 1994; Howerth *et. al.* 1988). Other domestic ruminants, such as cattle and goats, rarely show clinical symptoms. Following infection of BTV, cattle have prolonged viremia in which BTV nucleic acid can be present in red blood cells for 16-20 weeks (MacLachlan *et. al.* 1994). Therefore, cattle can serve as reservoir hosts for BTV.

There are 24 described serotypes of BTV worldwide; BTV has been found between latitudes 40ºN and 35ºS where suitable vectors are present (Tabachnick 1996). However, recent outbreaks in northern Europe have been recorded BTV as far north as Denmark, which is further north than 50 ºN (Saegerman *et. al* 2008). Until recently, five serotypes (2, 10, 11, 13, and 17) of bluetongue virus were known to occur in the United States (Mullen *et. al.* 1999). In November 2004, bluetongue virus serotype 1 was isolated for the first time in the U.S. from a hunter-killed deer in the marsh area of the Atchafalaya Delta in St. Mary Parish, LA (Johnson *et. al.* 2006). Previously, the known range of BTV serotype 1 was confined to the Caribbean and Central and South America (Tabachnick 1996). Prior to 2004 BTV serotypes 2, 10, and 17 were known to occur in Louisiana.

The only known vectors of BTV are biting midges of the genus *Culicoides*, family Ceratopogonidae. In the New World, the primary vectors of BTV are considered to be *C.*

sonorensis (Coquillett) in N. America and *C. insignis* (Lutz) in S. America and southern Florida (Mecham 2003; Tabachnick 2004; Blackwell 2004). The primary vector of bluetongue virus throughout most of the U.S. was considered to be *Culicoides variipennis* until Holbrook *et. al.* (2000) divided *C. variipennis* into 3 different species: *C. sonorensis, C. variipennis,* and *C. occidentalis*. Currently, the primary vector of BTV in the U.S. is considered to be *C. sonorensis* while *C. variipennis* and *C. occidentalis* are considered refractory to BTV infection (Tabachnick 2004).

Shortly after the BTV-1 positive deer was found, APHIS personnel conducted a serum survey of livestock herds at three nearby cattle farms, and found two of the farms to have BTV-1 seropositive cattle. The purpose of this study was to determine potential vectors of bluetongue viruses in areas of Louisiana where BTV-1 was discovered in the fall of 2004. One objective of this study was to compare the efficacy of different trap types for capturing specimens of coastal ceratopogonids in areas of Louisiana where BTV-1 seropositive deer and cattle had been identified. A second objective was to conduct assays to determine the presence and serotypes of BTV in pools of specimens of different ceratopogonid species collected during the procedures of the first objective.

CHAPTER 1. LITERATURE REVIEW

1.1 Biology and Ecology of Ceratopogonidae

There have been 125 genera and over 5500 species placed in the family Ceratopogonidae (order Diptera). These flies range in size from 1- 4mm and are considered to be some of the smallest flies in the world. Only four genera of ceratopogonids are known to contain haematophagous adult females: *Austroconops, Culicoides, Forcipoymia* subgenus *Lasiohelea,* and *Leptoconops* (Mellor *et. al.* 2000). Ceratopogonids are found throughout the world in association with many aquatic or semiaquatic habitats. Some common names of these flies include sand flies, punkies, no-see-ums, midges, and biting midges (Mellor *et. al.* 2000). In south Louisiana, they are more commonly referred to as gnats.

The life cycle of ceratopogonids is holometabolous, and includes the egg, four larval stages, pupa, and adult. Adult females usually lay eggs in batches; eggs hatch within two to seven days and are not resistant to drying (Meiswinkel *et. al.* 1994). Larvae require a certain amount of moisture and can be found in a wide range of habitats including edges of pools, streams, marshes, bogs, beaches, swamps, tree holes, irrigation pipe leaks, animal dung, and rotting fruit (Blanton and Wirth 1979). The development of larvae differs among species and the duration of each stage is dependent upon temperature. The larvae of certain species are predacious, feeding on nematodes, protozoa, immature insects, and other small aquatic organisms (Mullen 2002). Other species have larvae which feed on particles of vegetable matter. The overwintering stage for most species is the fourth-instar larvae in diapause (Kettle 1984). However, adults of some species are capable of overwintering, especially in areas with mild winters (Gerry and Mullens 2000; Khalaf 1969). Pupae are usually free floating but sometimes are

loosely attached to debris. The pupal stage duration usually lasts for two to three days,

but can last for several weeks depending upon species and temperature (Mellor *et. al.*

2000).

Most haematophagous female ceratopogonids are nocturnal, but some species are

diurnal. Specimens of *C. paraensis* are known to bite people during the day, and some

species in the genus *Austroconops* are day biters (Borkent and Craig 2004; Pinheiro *et. al.*

1981).   For most haematophagous *Culicoides*, a blood meal is required for egg

production. One study showed that *C. variipennis* takes a blood meal of 0.56 mg, which

is about 20% of a blood meal of a mosquito (Tempelis and Nelson 1971). There are

autogenous ceratopogonid species; the nutrition to produce eggs is provided by energy

reserves obtained as larvae (Blanton and Wirth 1979). Linley (1983) reported that 38

species of Ceratopogonidae are autogenous, including *C. furens*. Male ceratopogonids

feed exclusively on carbohydrates and do not have blood-feeding mouthparts.

Adult biting midges are usually short-lived and survive no longer than ten days,

but there are some exceptions with individuals living up to 90 days. Cribb (2000)

showed that members of the genus *Forcipomyia* can live up to 39 days after collection.

Goffredo *et. al*. (2004) reported that of 1,500 wild-caught midges from the *C. obsoletus*

complex, 3 lived for 92 days in the laboratory. Females that take more than one blood

meal are very important because flies must take at least two blood meals to transmit

pathogens that are not vertically transmitted. However, only a very small percentage of

female ceratopogonids are successful at getting a second blood meal (Mullen 2002).

1.2 Ceratopogonids as Vectors of Disease

There are over 1,400 species of *Culicoides* of which 96% are obligate blood-

feeders that attack mammals and birds (Meiswinkel *et. al.* 1994). Members of the genus

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Culicoides transmit pathogens to birds, humans, and animals and compromise the most important genus of the family Ceratopogonidae. Out of the 125 genera of Ceratopogonidae, only members of the genus *Culicoides* are known to be vectors of arboviruses. Worldwide more than 50 arboviruses have been isolated from *Culicoides* spp. (Mellor *et. al.* 2000) and some of these viruses are significant agents of disease.

Bluetongue virus (BTV) is the most economically important virus transmitted by members of the genus *Culicoides*. Bluetongue virus can affect all species of ruminants, and causes severe (often fatal) hemorrhagic disease in some species of sheep and deer. Mortality rates can be as high as 90% in infected whitetail deer and 70% in some susceptible breeds of sheep (The Center for Food Security and Public Health 2006). The largest known outbreak of bluetongue disease occurred between 1956-1960 when over 179,000 sheep died in Spain and Portugal (Gorman 1990). Bluetongue disease is classified as a notifiable multispecies disease by Office of International Epizootics because of its substantial economic impact and potential for rapid spread (OIE 2008). Members of the genus *Culicoides* are the only known competent vectors of BTV worldwide (Kramer *et. al.* 1985); different species of *Culicoides* are primary vectors of BTV around the world (Tabachnick 1996).

Epizootic hemorrhagic disease virus (EHDV) is very similar to BTV and also causes a severe hemorrhagic disease in ruminants. Epizootic hemorrhagic disease is considered to be the most important infectious disease of wild deer in the U.S. (Nettles *et. al.* 1991). The clinical signs in deer are identical for BTV and EHDV and virus isolation studies are required to differentiate the two viruses. The only known vectors of EHDV are flies in the genus *Culicoides*. In the U.S., the primary vector of EHDV is considered

to be *C. sonorensis* but other species of *Culicoides,* such as *C. lahillei* (= *C. debilipalpis*) have been suspected as vectors (Smith *et. al.* 1996).

African horse sickness virus (AHDV) causes a serious disease in equids that can cause up to 90% mortality in horses (Mellor 1993). The known vector of this virus is *C. imicola* (Capela *et. al.* 2003). The disease is mainly found in sub-Saharan Africa, but has occurred outside Africa on a few occasions, the most notable of which was a major outbreak in the Near and Middle East from 1959-1963 (Lubroth 1988).

Vesicular stomatitis, caused by vesicular stomatitis virus (VSV), is an infectious viral disease that primarily affects cattle, horses, and swine and can have devastating affects on the U.S. cattle industry. Vesicular stomatitis also can affect humans, especially when handling animals infected with VSV (Mead *et. al.* 2000). The modes of transmission of the virus are not fully understood, but in the U.S., VSV has been shown to infect salivary glands of *C. sonorensis* and biting midges are considered as potential vectors of VSV (Drolet *et. al.* 2005). Walton *et. al.* (1987) were the first to report VSV from field-collected *Culicoides*; VSV was isolated from specimens of *C. stellifer, C. variipennis,* and *C. (selfia)* spp.

Oropouche virus is the most important known human pathogen that is transmitted by biting midges in the genus *Culicoides*. The virus causes a disease similar to dengue fever in humans, and is transmitted by *C. paraensis*. Oropouche fever occurs in the Amazon region, Panama, and the Caribbean and is considered a major public health problem (Yanase *et. al.* 2005), causing more than half million cases in Brazil alone (Anderson *et. al.* 1961). Mercer *et. al.* (2005) showed what was thought to be a single species in Peru was actually two different species, *C. paraensis* and *C. insinuatus*, and his

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findings helped explain why the distribution of human cases of Oropouche did not correspond to the previous assumed range of *C. paraensis.*

Protozoan parasites in the genus *Haemoproteus* are known to be transmitted by *Culicoides* midges (Levine 1961). The parasite *Haemoproteus danilewski* causes a malaria-like disease in populations of wild birds which affects survival and reproduction of infected birds (Garvin *et. al.* 2003). Garvin and Greiner (2003) conducted a 2 year survey on the seasonal abundance of *Culicoides* spp. in south Florida and experimentally challenged the most abundant ornithophilic species with *H. danilewskyi.* The authors found three species (*C. edeni, C. knowltoni,* and *C. arboricola*) capable of supporting sporogonic development of *H. danilewskyi* and suggested that *C. edeni* was the most important vector of *H. danilewski.*

Onchocerca cervicalis* was a common filarial nematode of equines, which caused severe dermatitis in horses (Foil *et. al.* 1984; Rabalais *et. al.* 1974; Stannard *et. al.* 1975). The vectors of this filarid are biting midges in the genus *Culicoides* (Collins and Jones 1978).   In the U.S., the primary vector of *O. cervicalis* is considered to be *C. sonorensis* (Foil *et. al.* 1984) and the prevalence of equine onchocerciasis has been reported as high as 82.6% in horses in the gulf coast regions of Louisiana and Mississippi (Klei *et. al.* 1984). Foil *et. al.* (1987) showed that specimens of *C. sonorensis* and *O. cervicalis* skin microfilariae of infected ponies had corresponding peaks in south Louisiana.

1.3 Factors Affecting Ceratopogonids as Vectors of Disease

There is limited evidence to suggest that any arbovirus can be vertically or venereally transmitted in species of *Culicoides*. However, White *et. al.* (2005) did detect segments of BTV RNA in pools of larvae and pupae of both *C. sonorensis* and *C.*

crepuscularis, but they did not attempt to isolate any BTV. Therefore, to transmit a virus, the current school of thought is that biting midges have to take a viremic blood meal from a vertebrate host and then bite another host after the virus has replicated in the salivary glands (Mellor *et. al.* 2000). In competent arbovirus vectors, virus particles attach to gut cells in the hind of the midgut and begin to replicate (Eaton *et. al.* 1990). Virus particles then escape the midgut and enter the hemolymph where they infect secondary organs including the salivary glands. Virus particles then are released into the salivary ducts and are available for transmission during subsequent biting (Chandler *et. al.* 1985; Fu *et. al.* 1996).

A number of factors must be considered when studying members of the genus *Culicoides* and their ability to transmit viruses. For example, climate and weather can have a substantial impact on populations of *Culicoides*, and therefore outbreaks of disease transmitted by members of the genus *Culicoides.* During a two year study in East Baton Rouge Parish, La., greater than 97% of biting midges were collected from March to October when the mean daily temperature was between 10°C and 33°C (Sabio 2005). Rainfall can also play an important role in biting midge populations by increasing the number of available sites for development (Gerry and Mullens 2000). Temperature can have an impact on the replication rate of BTV and the survival of adult biting midges. At higher temperatures, infection rates and virogenesis are higher, but midges do not survive as long (Mullens *et. al.* 1995). Freezing temperatures can kill adult midges (Mellor *et. al.* 2000).

The seasonal distribution and abundance of *Culicoides* are very important to record when characterizing an epizootic of disease agents transmitted by biting midges (Mellor *et. al.* 2000). The seasonality of disease outbreaks is correlated with the timing

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of annual peaks for adult specimens of competent vectors of *Culicoides* (Mohammed and Mellor 1990). Establishing the occurrence of adults of *Culicoides* species during the winter months also is important in understanding how viral agents can overwinter (Mellor 1996). Sellers and Mellor (1993) suggested that some viruses transmitted by specimens of *C. imicola* can overwinter in flies in areas with a daily maximum temperature of at least 12.5°C in the coldest month of the year. Bluetongue virus can sometimes be transmitted by insects which are not normally considered vectors. For example, Mellor and Boorman (1980) showed that *C. nubeculosus* can be a vector for BTV when ingesting blood with both BTV and microfilariae of *Onchocerca cervicalis.*

Long range wind dispersal of adult biting midges has been documented and is considered to be important in the movement of arboviruses. Mellor *et. al.* (2000) referenced sixteen articles regarding long-range dispersal of *Culicoides* on winds. Murray (1987) presented convincing data associating Akabane disease outbreak in New South Wales with evidence of long-distance dispersal of *Culicoides brevitarsis* from the Hunter Valley. There was a severe drought in the area of the outbreak, and this area is also not included in the normal geographic distribution of *C. brevitarsis*. El Fatih *et. al.* (1987) also demonstrated proof for spread of BTV associated with prevailing winds in the Sudan. Strong wind events, such as hurricanes, are common in south Louisiana and wind dispersal of exotic species of *Culicoides* has been suggested. Recently, in 2007, experts at USDA's National Veterinary Services Laboratories (NVSL) announced that BTV serotypes 3, 5, 6, 14, 19, and 22 were isolated and identified from Florida (Stallnecht 2008). These BTV serotypes had never been reported in the U.S., and could be here as a result of long range wind dispersal of exotic vectors.

1.4 Bluetongue Virology and Disease

Bluetongue virus is a double-stranded RNA virus in the genus *Orbivirus*, family Reoviridae. The genome is made up of 10 genes which encode mRNAs for seven structural and three nonstructural proteins. The RNA genome is encapsulated in a double-layered protein coat (Roy *et. al.* 1990). Two major proteins, VP2 and VP5, are contained in the outer coat. The specificity of serotypes resides in the VP2 protein (Mecham *et. al.* 1986). Proteins VP3 and VP7 make up the inner coat and VP7 has been shown to be the protein involved in virus attachment (Xu *et. al.* 1997). An infectious sub particle is produced when VP2 is cleaved from the outer capsid and an inner core particle results from further enzyme treatment (Mertens *et. al.* 1987).

Bluetongue virus is thought to infect all known species of ruminants, and the World Organization of Animal Health (OIE) maintains that BTV is a transmissible disease that has the potential for serious, rapid spread, and is of major importance in the international trade of animals and animal products. There are international regulations that prohibit the movement of livestock and relative products from BTV endemic areas to BTV-free areas, and these regulations create indirect losses for livestock producers (Blackwell 2004). Tatem *et. al.* (2003) indicated that the economic impact of BTV was in the order of 3 billion USD per year worldwide. Bluetongue virus has been isolated in bull semen, and heifers have been shown to contract BTV through insemination with BTV-infected sperm (Bowen and Howard 1983; Luedke *et. al.* 1977).

Bluetongue disease, which was first reported in South African sheep (Hutcheon 1902), can cause severe morbidity and mortality in sheep of certain breeds and deer of some species (Mellor *et. al.* 2000). Some common symptoms of bluetongue disease include fever, lameness, oral lesions, swollen muzzle, necrosis of the tongue ("blue

tongue"), and hemorrhaging of the coronary bands of infected adults. Early prenatal

infection in cattle can lead to embryonic death resulting in abortions or still births

(Tabachnick 1996). Less than 5% of infected adult cattle show any clinical signs, but

cattle can develop a prolonged viremia lasting several weeks, which makes cattle ideal

reservoir hosts for BTV.

1.5 BTV Serotype Distribution, Vectors of BTV, and Vector Competence

Currently, there are 24 different recognized serotypes of bluetongue virus (genus

Orbivirus; family Reoviridae) distributed differentially worldwide (Tabachnick 1996).

In the Central American-Caribbean Basin, BTV serotypes 1, 3, 4, 6, 8, 12, 14, and 17

have been observed (Tanya *et. al.* 1992; Thompson *et. al.* 1992). In Australia, where

BTV serotypes 1, 3, 9, 15, 16, 20, 21, and 23 are transmitted by *C. wudui, C. brevitursis,*

C. fulvus, and C. ucfoni, there is not much clinical disease in ruminants (Tabachnick

1996). Serotypes 1, 2, 3, 9, 12, 14, 19, 20, 21, and 23 occur in Asia where several species

of *Culicoides* are known to be vectors (Taylor 1986). Serotypes 1-19, 22, and 24 are

found in Africa and the Middle East, where the primary vector is C. *imicola* (Mellor

1990). In many parts of the world, BTV vectors are unknown due to lack of research on

the subject (Tabachnick *et. al.* 1992).

Recently, BTV-8 has caused a severe epizootic in northern Europe. Before 1998,

bluetongue disease was considered to be an exotic disease in Europe. From 1998

through 2005, 5 serotypes of BTV (1, 2, 4, 9, and 16) were detected in the

Mediterranean Basin (Saegerman *et. al.* 2008). The suspected vector of BTV in Europe

was *C. imicola* (primary BTV vector in Asia and Africa), which has now been recorded

as far north as 44°N (Goffredo *et. al.* 2001). However, in the region of the outbreak in

2006, a pool of 50 nonengorged, parous *C. dewulfi* (Goetghebuer) collected in the

Netherlands were positive by PCR for BTV (Meiswinkel *et. al*. 2007). Also, Savini *et. al.* (2004) isolated BTV from field collected specimens of the *Culicoides obsoletus* complex in central Italy. Furthermore, BTV was isolated from field-collected specimens of *C. pulicaris* in Sicily (Caracappa *et. al.* 2003). Therefore, it has been suggested that many species of *Culicoides* in Europe could be competent vectors of BTV.

Until the discovery of BTV-1 in south Louisiana in 2004 (Johnson *et. al.* 2006), 5 of the 24 serotypes of BTV were known to occur in the U.S. (Mullen *et. al.* 1999); serotypes 2, 13, and 17 were known to occur in Louisiana (Wieser-Schimpf *et. al*. 1993). In 2007, experts at USDA's National Veterinary Services Laboratories announced that BTV serotypes 3, 5, 6, 14, 19, and 22 were isolated and identified from Florida (Stallnecht 2008). This was the first report of these six serotypes in the U.S. The distribution of bluetongue viruses worldwide corresponds to the distribution of *Culicoides* vectors (St. George and Peng 1996). The only proven vectors of BTV are members of the genus *Culicoides* (Kramer *et. al*. 1985; Ward 1996; Ward 1994; Hoar *et. al*. 2004). Worldwide, at least seven species of *Culicoides* are considered as major vectors of BTV although many species are considered possible vectors (Paweska *et. al*. 2002; Tabachnick 2004). In the Central American-Caribbean Basin, the most likely BTV vector is *C. insignis* (Tanya *et. al.* 1992)*.* In Australia, BTV is transmitted by *C. wudui, C. brevitursis, C. fulvus,* and *C. ucfoni* (Tabachnick 1996), and in Africa and the Middle East the primary vector is C. *imicola* (Mellor 1990). In Asia, it is uncertain which species of *Culicoides* are BTV vectors (Mellor *et. al.* 2000). In the United States, species of the *Culicoides variipennis* complex are the major vectors of BTV (Tabachnick 1996). Until Holbrook *et. al*. (2000) clarified that what we thought was one species (*C. variipennis*) was actually three species (*C. sonorensis, C. occidentalis,* and

C. variipennis), it was believed that there were geographic populations of *C. variipennis* that were refractory to BTV infection.

In the U.S., bluetongue virus was first isolated from sheep in California in 1952 (McKercher *et. al.* 1953). Foster *et. al.* (1963) demonstrated that *C. variipennis* was a biological vector of BTV after feeding specimens on infected sheep, incubating the specimens for 10-15 days, and then allowing them to feed on non-infected sheep, which became infected. The authors also isolated BTV via cell culture from specimens of *C. variipennis*. Jochim *et. al.* (1966) first showed that BTV replicated in specimens of *C. variipennis* inoculated with BTV, and Bowne and Jones (1966) showed that BTV replicated in the salivary glands of *C. variipennis*. Kramer *et. al.* (1990) isolated BTV-11 using cell culture techniques from wild-caught *C. variipennis* in Colorado and Utah. Wieser-Schimpf *et. al.* (1993) reported BTV serotypes 2, 13, and 17 from seropositive cattle in Baton Rouge, La., and found 1 out of 135 pools of *C. variipennis* to be positive for BTV via PCR (the serotype was not reported).

Currently, *Culicoides sonorensis*, which occurs in the southern and southwestern U.S., is considered to be the principal vector of BTV in the United States because of vector competence and field studies; however, *C. insignis* also is known to transmit BTV-2 in southern Florida (Mecham 2003; Holbrook and Tabachnick 1995; Tabachnick 1996; Tabachnick 2004). The other two species from the *C. variipennis* complex (which are refractory for BTV) are *C. variipennis*, which occurs mostly in the East and South and *C. occidentalis,* which occurs in the Southwest (Holbrook *et. al.* 2000; Tabachnick 1996). Therefore, it should be noted that in discussions of references to work conducted before the publication of Holbrook *et. al.* (2000) that *C. variipennis* is a likely synonym for C. *sonorensis* for vector competence studies. Specimens of *C. variipennis* are not

considered vectors of BTV because they have a low susceptibility rate to infection in the lab, and no virus has been isolated in field-collected specimens (Tabachnick 1996). Specimens of *C. occidentalis* collected at Borax Lake in California also had low susceptibility rates for BTV and no BTV has been isolated from flies of this species (Tabachnick 1996).

The epidemiology of BTV transmission in the U.S. with *C. sonorensis* as the primary vector has been generally accepted (Holbrook and Tabachnick 1995; Tabachnick 1996; Tabachnick 2004). However, BTV-2 was first isolated in Florida in 1982 and is thought to be transmitted solely by *C. insignis*, although *C. sonorensis* is also present in Florida (Mecham 2003). Greiner *et. al.* (1985) isolated BTV-2 from field collected specimens of *C. insignis* in Ona, Florida and Tanya *et. al.* (1992) showed that specimens of *C. insignis* were competent biological vectors of BTV. In Louisiana, an association of transmission of BTV-13 and BTV-17 with seasonal peaks of *C. variipennis* was shown, and also BTV RNA was found in one of 381 pools (6,072 flies) of *C. variipennis* via PCR (Wieser-Schimpf *et. al.* 1993).

1.6 Epizootic Hemorrhagic Disease Virus and Disease

Epizootic hemorrhagic disease virus (EHDV) is a double stranded RNA *Orbivirus* composed of 10 dsRNA segments (Huismans *et. al.* 1979; Mecham and Dean 1988). The genome codes for three nonstructural and seven structural proteins. Genome segment 2 codes for the major viral protein and is associated with serotype specificity and induction of neutralizing antibody (Mecham and Dean 1988). Genome segments 1, 3, 4, 6, and 8 are highly conserved with over 90% homology among cognate genes of other EHDV serogroups (Wilson *et. al.* 1990). Shope *et. al.* (1960) first isolated EHDV serotype 1 after a whitetail deer die off in 1955 in New Jersey.

Epizootic hemorrhagic disease virus is very similar to BTV morphologically, however they differ antigenically. Another difference between the two viruses is that BTV causes disease in sheep whereas EHDV does not cause disease in sheep (Fletch and Karstad 1971). The clinical disease and symptoms in deer are identical for BTV and EHDV, and only virus isolations can differentiate between the two viruses. "Hemorrhagic disease" is the collective term often used to describe disease caused by BTV or EHDV. Deer with epizootic hemorrhagic or bluetongue disease can develop severe hemorrhaging in major organs such as the spleen and liver which leads to death. Clinical signs of epizootic hemorrhagic or bluetongue disease include anorexia, weakness, nasal mucosa, salivation, and sometimes necrosis on the coronary bands (Fay *et. al.* 1956). The pathogenesis of epizootic hemorrhagic or bluetongue disease results from vascular endothelial cell damage due to viral replication in these cells (Tsai and Karstad 1973).

1.7 EHDV Serotype Distribution, **Vectors, and Vector Competence**

At least 10 serotypes of EHDV are distributed worldwide (Gorman 1992). Currently, little is known about the global epidemiology of EHDV (Aradaib and Ali 2004). In the U.S., the occurrence of EHDV serotypes 1 and 2 were described by Foster *et. al.* (1977). Up until 2006, EHDV-1 had been isolated from Mississippi and Missouri and EHDV-2 had been isolated from deer from Colorado, Georgia, Illinois, Kansas, Louisiana, Missouri, and Texas (Stallnecht 2006). In 2006, EHDV-6 also was described in white-tail deer in Illinois and Indiana (Stallnecht 2008). In Nigeria, serotypes 3 and 4 were isolated from *Culicoides spp* (Moore 1974). In Australia, 5 serotypes have been identified: EHDV-5 through EHDV-8 (Aradaib and Ali 2004).

The only known vectors of EHDV are biting midges in the genus *Culicoides*. In Africa, EHDV has been isolated from midges in the *C. schultzei* group and in Australia the virus has been isolated in *C. brevitarsis* (Parsonson and Snowdon 1985). In Sudan, the primary vector of EHDV is *C. imicola* (Aradaib *et. al.* 1999), but the vectors of EHDV are unknown in Central America, South America, Japan, and Southeast Asia (Mellor *et. al.* 2000).

In the U.S., the primary vector of EHDV is thought to be *C. sonorensis*. Jones *et. al.* (1977) isolated EHDV from parous specimens of *C. sonorensis* in Kentucky captured with modified CDC light traps. Foster *et. al.* (1977) showed that two different strains (NJ and KY) of EHDV could be transmitted by wild-caught *C. sonorensis*; these two strains of EHDV were eventually named EHDV-1 (NJ) and EHDV-2 (KY). Furthermore, Foster *et. al.* (1980) reported that both strains of EHDV were isolated from cattle and wild-caught specimens of *C. sonorensis* during the same period of time. More recently, Smith *et. al.* (1996c) isolated EHDV-2 from specimens of both *C. lahillei* (*C. debilipalpis*) and *C. variipennis* that had been fed on viremic deer and then tested 4-15 days later. In California, Rosenstock *et. al.* (2003) tested wild-caught specimens of *C. mohave* for EHDV using PCR, and reported that 35% of the pools tested positive for EHDV.

CHAPTER 2. COMPARING TRAP EFFICACY OF THREE DIFFERENT TRAP TYPES FOR CAPTURING SPECIMENS OF COASTAL *CULICOIDES* IN LOUISIANA

2.1 Introduction

Bluetongue virus (BTV) is known to infect many different domestic and wild ruminants. Infection with BTV can cause serious disease with high mortality rates in sheep and deer, but infected cattle are normally asymptomatic. Bluetongue disease is a notifiable multispecies disease by the Office of International Epizootics because of its substantial economic impact and potential for rapid spread (OIE 2008). There are 24 serotypes of BTV worldwide; Tabachnick (1996) indicated that BTV had been found between latitudes 40ºN and 35ºS wherever suitable vectors were present. However, recent outbreaks in northern Europe have recorded BTV as far north as Denmark, which is further north than 50 ºN (Saegerman 2008). Until recently, five serotypes (2, 10, 11, 13, and 17) of bluetongue virus were known to occur in the United States (Mullen *et. al.* 1999). In November 2004, BTV serotype 1 was isolated from a deer shot in the marsh area of the Atchafalaya Delta in St. Mary Parish, LA (Johnson *et. al.* 2006). This was the first report of BTV serotype 1 in the United States; previously the New World distribution of BTV serotype 1 included the Caribbean and Central and South America.

Biting midges (genus *Culicoides*, family Ceratopogonidae) are the only known vectors of bluetongue virus (Kramer *et. al.* 1985). In the New World, the primary vectors of BTV are considered to be *C. sonorensis* (Coquillett) in N. America and *C. insignis* (Lutz) in S. America (Tabachnick 2004; Blackwell 2004). The primary vector of bluetongue virus in the U.S. was considered to be *Culicoides variipennis* until Holbrook *et. al.* (2000) divided *C. variipennis* into 3 different species: *C. sonorensis, C. variipennis,* and *C. occidentalis*. The primary vector of BTV in the U.S. is considered to

be *C. sonorensis*, while *C. variipennis* and *C. occidentalis* are considered to be refractory to BTV infection.

There have been many attempts to determine the most effective trap type for capturing specimens of *Culicoides variipennis*. Barnard and Jones (1980) showed that the greatest number of *C. variipennis* occurred during the full moon and that flight activity increased during moonlight hours; the greatest diel activity was near sunset but sometimes increased near sunrise. Rowley and Jorgensen (1967) showed that New Jersey traps modified with a black light caught almost 11 times more specimens of *Culicoides* spp. than a New Jersey trap with the standard incandescent 40W bulb. Holbrook (1985) reported that black light miniature CDC traps (Model 512, John W. Hock Co., Gainesville, FL 32604) baited with dry ice caught 17 times more *C. variipennis* than traps without dry ice. Holbrook and Bobian (1989) compared the efficacy of six different trap types (standard New Jersey trap with incandescent 40W bulb with dry ice, New Jersey trap without dry ice, a standard ABADRL baffle trap without dry ice, baffle trap with dry ice, a CDC trap with dry ice and no light, and a 12 V updraft trap with two 5 w light bulbs) for capturing parous female specimens of *C. variipennis*. The authors found that the New Jersey trap without dry ice caught the highest proportion of parous *C. variipennis*, and therefore, recommended the use of the standard New Jersey trap for capturing specimens of *C. variipennis* for virus assays. Kramer *et. al.* (1985) captured large numbers of *C. insignis* in Florida using both New Jersey traps with incandescent light and black light CDC traps baited with dry ice.

Wieser-Schimpf *et. al.* (1990) showed that a New Jersey trap modified with a 15 W black light caught 10 times more parous empty and gravid females of *C. variipennis* than the New Jersey trap with an incandescent bulb in Louisiana. The purpose of this

study was to determine which species of *Culicoides* were present in the coastal area where the BTV-1 positive deer was found and on nearby cattle farms. Wieser-Schimpf *et. al.* (1990) compared different trap types for capturing specimens of *C. variipennis* on inland farms, but there have been no studies to find the most efficient trap type for capturing coastal midges in the genus *Culicoides* in Louisiana. Therefore, one objective of this study was to compare the efficacy of different trap types for capturing specimens of coastal ceratopogonids. A second objective was to compare the ceratopogonid populations of the Atchafalaya Delta marsh area and the nearby cattle farms with BTV-1 seropositive cattle.

2.2 Materials and Methods

- Farm Trap Study

Three cattle farms (two of which had BTV-1 seropositive cattle; see Chapter 3) were chosen in St. Mary Parish within 30 km of the location where the positive BTV-1 deer was shot (Appendix). There were two trap sites greater than 100 m at each of the three farms. All farms had some type of standing water in ditches or canals surrounding the pastures, which occasionally filled with floodwater from heavy rain events. Farm A (owned by Wayne Cantrell) had approximately 820 km^2 of pastures containing 127 head of cattle. The pastures were surrounded by bottomland hardwood forests. This farm contained low lying marsh habitat and was located approximately 60 km north of the Gulf of Mexico. The GPS coordinates for the two trap sites at Farm A were: 29.67237N -91.27583W and 29.67275N -91.27821W. Farm B (owned by Joe Trahan) was located 2.19 km southwest of Farm A and had 215 km^2 of pastures with 72 head of cattle. This farm also was surrounded by bottomland forest. The GPS coordinates for the two trap sites at Farm B were: 29.654885N -91.284511W and 29.654842N - 91.287324W. Farm

C (owned by Kevin Brightwell) was located 11.49 km northwest of Farm B and contained 134 km^2 of pastures with 21 head of cattle. The GPS coordinates for the two trap sites at Farm C were: 29.70298N -91.38673W and 29.70402N -91.38728W.

Traps were hung from tree branches approximately 1.5-2.0 m above ground, and one trap at each farm was baited with 2 kg of dry ice. Traps were deployed twice per month from January 1, 2006 through November 15, 2007 and the dry ice was rotated between the two sites at each trap at each farm. The traps were set out before dusk and retrieved after sunrise. Sealed, gelled-electrolyte six volt twenty amp hour rechargeable batteries (model 2.32; John W. Hock Co., Gainesville, FL) were used to power the traps and double ring fine mesh collection bags (model 1.45; John W. Hock Co., Gainesville, FL) were used on the CDC traps to collect insects. The nets were collected and stored in a dry ice container with approximately 15 kg of dry ice. Subsequently, the nets were transported to LSU and transferred into an ultra cold freezer at -80° C.

Nets were emptied into a large Petri dish and insects were sorted on a chill table (BioQuip®, Gardena, CA) using a dissecting microscope. All ceratopogonids were separated into genus, and the specimens in the genus *Culicoides* were furthered sorted by species. Specimens were sorted by examining the wing veination, number of antennal segments, spermathecae, and maxillary palps using keys in the Manual of Nearctic Diptera (1981) and of Blanton and Wirth (1979). Specimens were separated by species, site, and date and placed into labeled 1.5 ml vials. The number of specimens of 3 species of *Culicoides* caught per trap-night in CDC traps with and without dry ice were compared using Student's t- test (SAS Institute 2000) and tested for significant differences (alpha = 0.05 level). Also, Shannon's equitability (E_h) was computed for 2006 and 2007 using Microsoft Excel 2007 (Begon *et. al.* 1996; Weaver and Shannon 1949).

- Intensive Farm Trap Study

An intensive trap study trial was conducted at farms A and C from May 22 to June 6, 2007, and a second trial was conducted at farms A and B from August 21 to September 3, 2007. Three traps types were used: 1) New Jersey Stainless Steel Light Trap with a 40W incandescent light bulb (model 1112; John W. Hock Co., Gainesville, FL), 2) New Jersey Stainless Steel Light Trap modified with black light, and 3) miniature CDC black light trap baited with 2 kg of dry ice. The New Jersey trap was modified with a black light according to Wieser-Schimpf *et. al.* (1991) except we used a 120V 60Hz ballast (Lot # LQ206FTP; Advance) and two F8T5 8W black light bulbs. Where 110 V alternating current was not available, a rechargeable sealed AGM 12 volt, 100 amp hour battery and a 600 watt DC to AC power inverter was used to power the New Jersey Traps. The 12 volt battery and power inverter were placed in a dry, sealed toolbox. The same two sites at the farms from the previous study were used in addition to a third site that was greater than 100 m from the other two sites. The GPS coordinates for the additional sites on Farm A, B, and C were, 29.668474N -91.270161W, 29.655748N -91.285463W, and 29.705578N -91.389353W, respectively.

All traps were hung from tree branches approximately 1.5-2.0 m above the ground and double ring, fine mesh collection bags were used on all traps. The traps were rotated daily three times per week for three consecutive weeks for 27 trap nights per farm for a total of 54 trap nights for each of the two trials. Nets were collected after sunrise and stored in a dry ice container until being transported to LSU campus. The nets were stored at -80 ºC and insects were processed by the methods described above. The mean number of specimens per trap-night for each of the three trap types was compared using ANOVA and Tukey's test for separation of means (SAS Institute 2000).

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- Atchafalaya Delta Trap Study

Seven sites were chosen in the Atchafalaya Delta Area in the immediate area where the BTV-1 positive deer was shot in 2004. The Atchafalaya Delta Area is a 57,060 hectare area located at the mouths of the Atchafalaya River and the Wax Lake Outlet in St. Mary Parish. The area is located approximately 40 km south of the towns of Morgan City and Calumet. Approximately 2,456 hectares of marsh and scrubby habitat occur on the main delta.

Miniature CDC black light traps were deployed once per month from January 2006 to November 2007 by Louisiana Department of Wildlife and Fisheries personnel before sunset and picked up after sunrise. The trap sites were only accessible by boat, and traps were hung from trees or structures approximately 1.5-2.0 m off the ground. Four trap sites were located on the Wax Lake Outlet Delta (including the exact location where the BTV-1 positive deer was shot), and the other three were located on the Atchafalaya River Delta. The GPS coordinates for the seven sites were 29.44879N -91.3372W, 29.52216667N -91.41548333W, 29.5279N -91.4046W, 29.45446N -91.32124W, 29.4478N -91.33881W, 29.52896667N -91.4073W, and 29.52485N -91.42018333W.

After collection, the nets were transferred to LSU and stored at -20° C. Using a dissecting microscope, specimens of ceratopogonids were sorted into genus and members of the genus *Culicoides* further into species using the keys of Blanton and Wirth (1979) and the methods described above. Shannon's equitability (E_h) was computed using Microsoft Excel 2007 for 2006 and 2007 and compared to the results of the farm trap study (Begon *et. al.* 1996; Weaver and Shannon 1949).

2.3 Results

▪ Farm Trap Study

In 2006, a total of 590 ceratopogonids were captured on the farms with CDC black light traps with and without dry ice. Of that total, 41% were of the genera *Forcipomyia* (182) or *Atrichopogon* (60). No ceratopogonids were caught in January 2006. In February, only 2 specimens of *Culicoides crepuscularis* were caught. From March until December 2006, specimens of eight species of the genus *Culicoides* were captured. The species were: *C. arboricola* (127), *C. debilipalpis* (123), *C. crepuscularis* (53), *C. haematopotus* (18), *C. paraensis* (10), *C. hinmani* (7), *C. stellifer* (6), and *C. furens* (4). The most ceratopogonids (208) caught in one month were captured in August. The species of which the most specimens were captured was *C. arboricola*, which represented 21.5% of the total specimens captured. The second most frequently captured species was *C. debilipalpis*, which represented 20.8 % of the total.

In 2007, a total of 1,078 ceratopogonids were captured on the farms using CDC black light traps with and without dry ice. Of this total, 40.6 % were of the genera *Forcipomyia* or *Atrichopogon*. No ceratopogonids were caught in January, November, or December. Ten species of *Culicoides* were caught throughout the year: *C. arboricola* (283), *C. debilipalpis* (211), *C. crepuscularis* (91), *C. haematopotus* (25), *C. paraensis* (16), *C. hinmani* (6), *C. stellifer* (4), *C. biggutatus* (2), *C. nanus* (1), *C. furens* (1). Again, the most specimens captured of any species was *C. arboricola* (26.3%) and the second most frequently caught was *C. debilipalpis* (19.6%).

Over the two year farm trap study, CDC light traps baited with dry ice caught significantly more flies than CDC traps without dry ice for three species which accounted for over 80% of the specimens of *Culicoides* captured: *C. arboricola*, *C. debilipalpis*, and

C. crepuscularis (Figure 2.1). Shannon's equitability was 0.64 for 2006 and 0.63 for

2007.

- Intensive Farm Trap Study

In the first intensive trap study, a total of 350 specimens representing 8 species of

Culicoides were captured. The CDC traps with dry ice caught specimens of eight species

(*C. arboricola*, *C. debilipalpis, C. crepuscularis, C. haematopotus, C. paraensis, C.

hinmani*, *C. stellifer* and *C. furens*). The New Jersey trap modified with black light

caught specimens of 5 of the 8 species, excluding C. *debilipalpis, C. hinmani,* and *C.

stellifer.* The New Jersey trap with an incandescent light only caught specimens of two

species: *C. arboricola* and *C. crepuscularis.* The CDC trap baited with dry ice caught

significantly more specimens of *C. arboricola, C. crepuscularis,* and *C. debilipalpis* than

the two New Jersey traps (Table 2. 1). There were no significant differences in the mean

number of specimens caught among all three trap types for *C. furens* and *C.

haematopotus.*

For the second intensive trap study, a total of 452 specimens representing 7

species of *Culicoides* were captured. The CDC trap with dry ice caught specimens of all

seven species which were: *C. arboricola*, *C. debilipalpis, C. crepuscularis,

C.haematopotus, C. paraensis, C. hinmani*, and *C. stellifer.* The New Jersey trap with

black light caught specimens of 4 species: *C. arboricola*, *C. debilipalpis, C.

crepuscularis,* and *C. haematopotus.* The New Jersey trap with an incandescent light also

caught specimens of 4 species: *C. arboricola*, *C. debilipalpis, C. haematopotus,* and *C.

hinmani.* There were no significant differences in the mean number of specimens for all

three trap types for *C. arboricola* and *C. haematopotus* (Table 2. 2). There was a

significant difference in the mean number of specimens for *C. debilipalpis* between the
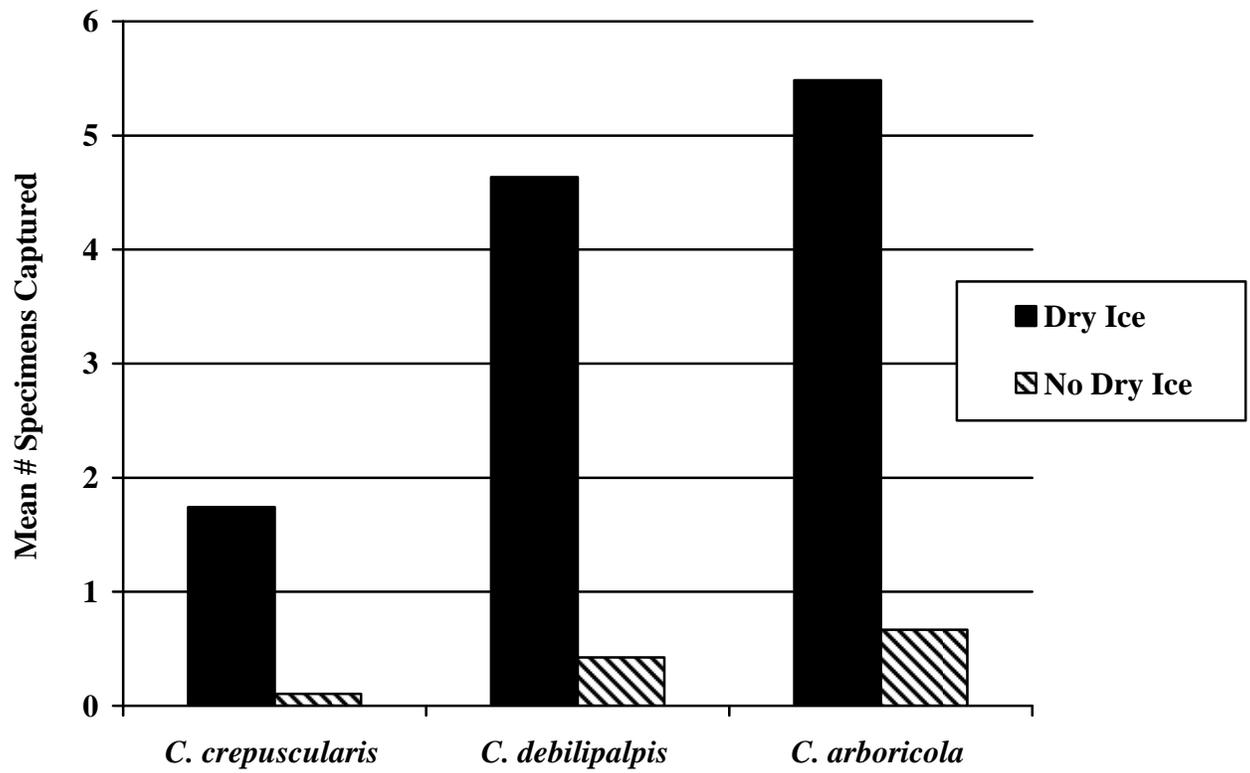
Figure 2. 1. Mean number of specimens of three species of *Culicoides* captured with CDC traps with and without dry ice at three cattle farms from January 2006 to November 2007 in St. Mary Parish, La.
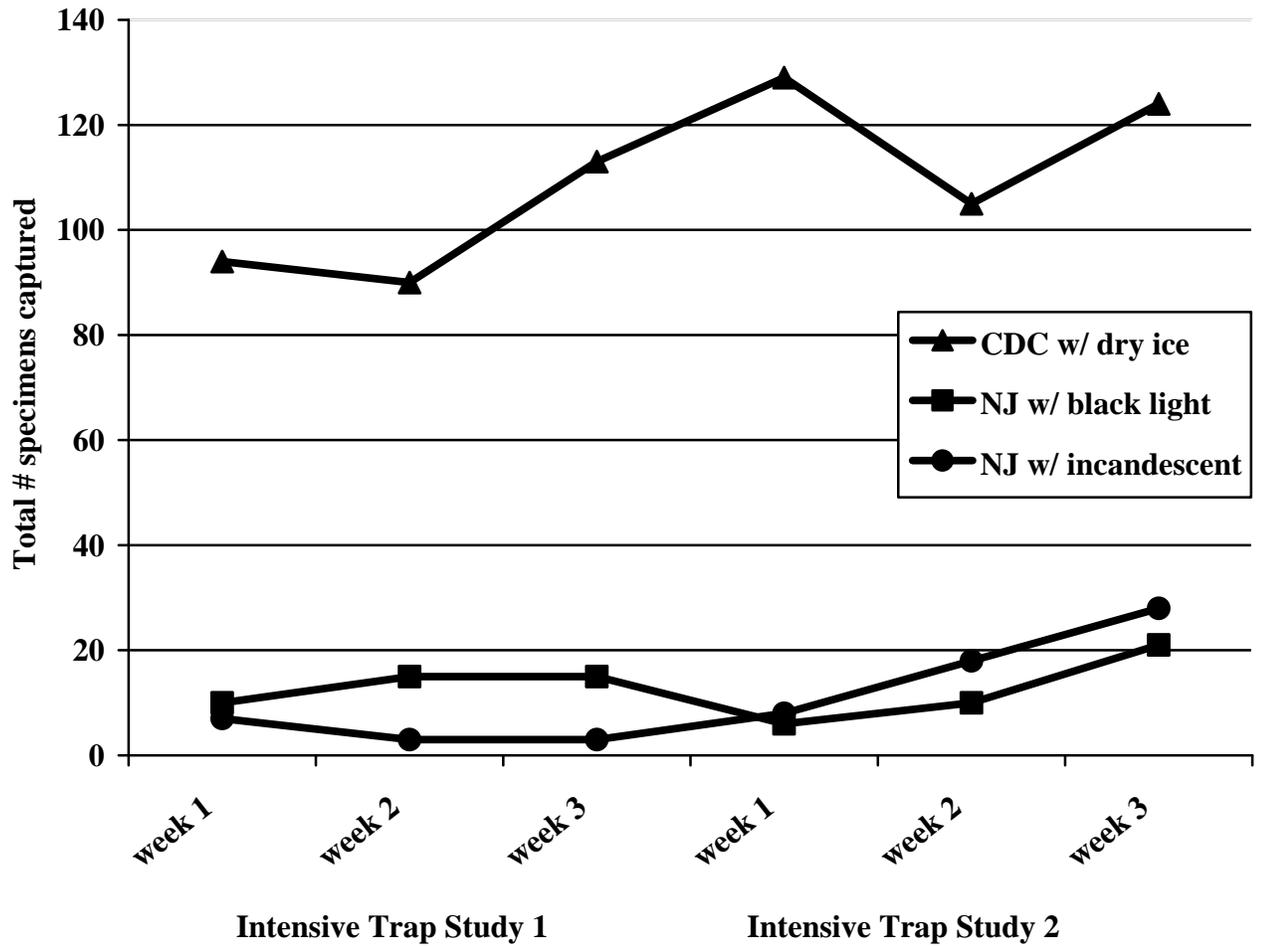
Figure 2. 2. Total number of specimens of *Culicoides* species captured with three different trap types at three cattle farms in 2007 in St. Mary Parish, La.

Table 2. 1. The mean number of specimens of five species of ceratopogonids for three trap types captured at farms A and C from May 22 to June 6, 2007 in St. Mary Parish, La.

Species	CDC w/ Dry Ice	NJ w/ Black Light	NJ w/ Incandescent
C. arboricola	6.0a	1.0b	0.3b
C. crepuscularis	7.9a	1.0b	0.1b
C. debilipalpis	4.3a	0.0b	0.0b
C. furens	0.9a	0.2a	0.0a
C. haematopotus	0.4a	0.1a	0.0a

After testing by ANOVA and Tukey's separation of means, values across rows followed by the same letter were not significantly different (P > 0.05).

Table 2. 2. The mean number of specimens of four species of ceratopogonids for three trap types captured at farms A and B from August 21 to September 3, 2007 in St. Mary Parish, La.

Species	CDC w/ Dry Ice	NJ w/ Black Light	NJ w/ Incandescent
C. arboricola	5.8a	1.6a	2.7a
C. crepuscularis	1.4a	0.4ab	0.0b
C. debilipalpis	10.6a	0.3b	0.2b
C. haematopotus	0.5a	0.3a	0.1a

After testing by ANOVA and Tukey's separation of means, values across rows followed by the same letter were not significantly different (P > 0.05).

CDC with dry ice and the two New Jersey traps. For *C. crepuscularis,* there was a significant difference between the mean number of specimens between the CDC with dry ice and the New Jersey trap with incandescent light.

▪ Atchafalaya Delta Trap Study

In 2006, a total of 1,699 ceratopogonids were captured in the Atchafalaya Delta area from March until November, and none were caught in January, February, or December. Of these, *Atrichopogon* (308)*, Forcipomyia* (143), and 5 species of *Culicoides* [*C. crepuscularis* (1,166)*, C. arboricola* (65)*, C. furens* (11)*, C. hinmani* (4)*, C. debilipalpis* (2)] were identified. Almost 75% of the total ceratopogonids captured in 2006 were *C. crepuscularis*, which had a large population peak in April.

In 2007, no ceratopogonids were caught in January, February, November, or December. From March until October 2007, a total of 3,161 ceratopogonids were captured and 64.2% of them were in the genus *Culicoides* representing 5 species including: *C. crepuscularis* (1974 - 62.4%)*, C. furens* (33 - 1.0%)*, C. arboricola* (20 - 0.6%)*, C. haematopotus* (2 – 0.06%)*, C. paraensis* (1 – 0.03%). Specimens of two other genera of Ceratapogonidae were caught in the traps: *Forcipomyia* (890-28.2%) and *Atrichopogon* (241 - 7.6%). The most ceratopogonids (1,550) were captured in April. The most specimens captured of any species was *C. crepuscularis,* which represented 62.4% of the total ceratopogonids captured and 97.2% of specimens of the genus *Culicoides*. Shannon's equitability was 0.14 for 2006 and 0.08 for 2007.

2.4 Discussion

A total of 988 specimens representing 10 species of the genus *Culicoides* were captured in the farm trap study. Khalaf (1966b) reported a total of 22 species of *Culicoides* captured in 30 widely distributed locations in south Louisiana using light-

traps. Some of the collections made by Khalaf (1966b) were from Berwick, Cypremort Point, New Iberia, Cote Blanche, and Weeks Island, and all of these locations are in the general coastal area of the farm trap study. Of the ten species we captured in the farm study, nine of them were reported by Khalaf (1966b), who did not capture specimens of *C. debilipalpis*.

For the farm trap study, we caught significantly more specimens of the genus *Culicoides* with CDC traps with dry ice than traps without dry ice. Nelson (1965) demonstrated that female specimens of *C. variipennis* are attracted to traps baited with dry ice after it was speculated by Reeves (1951) that CO_2 might play a role in host-seeking patterns of *Culicoides* spp.. Furthermore, Holbrook (1985) showed that CDC traps baited with dry ice caught 17 times more specimens of *C. variipennis* than traps without dry ice. In this study, we did not capture any specimens of *C. variipennis*. However, the same pattern of catching more specimens of *Culicoides* spp. with the addition of dry ice was observed. For example, we captured almost 11 times more specimens of *C. debilipalpis* in CDC traps baited with dry ice than traps without dry ice. Holbrook and Bobian (1989) recommended using New Jersey traps without dry ice for capturing the highest proportions of parous females of C. *variipennis* for conducting virus assays. However, visual markers such as the abdominal tergite pigmentation of parous *C. variipennis* are not prominent for all species of *Culicoides.* Since we did not know which species occurred in the study area, we tested the efficacy of different trap types for capturing higher numbers of flies. We found that black light CDC traps with dry ice caught significantly more specimens of *Culicoides* of the southern coast of La. than CDC traps without dry ice. This is important information because when trapping is to be done

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in a new area where there is an outbreak of BTV, it is necessary to use the most efficient

trapping method for capturing potential insect vectors.

We also found that CDC traps with dry ice caught significantly more specimens

of *Culicoides* spp. than New Jersey traps with incandescent or black light. There have

been many studies to determine the most efficient trap type for capturing specimens of

Culicoides. Anderson *et. al.* (1989) compared seven different trap types and found that

the CDC trap with a black light and baited with dry ice caught significantly more

specimens of *C. variipennis* than all other traps. In our study, we compared three

different trap types: CDC black light trap with dry ice, New Jersey trap with incandescent

light, and a modified New Jersey trap with black light. Overall, the CDC trap caught 4.7

times more *Culicoides* midges than the two New Jersey traps combined (Figure 2. 2).

For the second intensive trap study, the CDC trap with dry ice caught over 21 times more

specimens of *C. debilipalpis* than the other two traps combined. We can therefore

recommend using CDC black light traps with dry ice for capturing the highest number of

biting midges in the genus *Culicoides* in the coastal area of La.

In the Atchafalaya Delta Trap study, we captured a total of 3,278 specimens of

Culicoides representing 7 species. The species diversity was lower in this area than at the

farms. This may be due to a larval habitat difference between the areas, such as high

salinity or pH levels, which inhibit the survival of larvae of certain species of *Culicoides*.

Also, Shannon's equitability was lower in both years for the Atchafalaya traps than the

Farm traps; Shannon's equitability explains the distribution of individuals among species

in an area and assumes a value between 0 and 1 with 1 being complete evenness (Begon

et. al. 1996). Therefore, as Shannon's equitability approaches 1, the individuals in the

population are more evenly distributed among species. Shannon's equitability for the

Atchafalaya Delta was 0.14 for 2006 and 0.08 for 2007; for both years, the number was closer to 0 than 1 meaning that the distribution of individuals among species was skewed. The low Shannon's equitability value for the Atchafalaya Delta area may be explained by the fact that there was one dominant species, *C. crepuscularis*, which accounted for over 95% of the total *Culicoides* specimens captured. On the other hand, Shannon's equitability for the farms was 0.64 for 2006 and 0.63 for 2007; on the farms, we captured specimens of 10 *Culicoides* species and the individuals were more equally distributed among species (Shannon's equitability closer to 1).

Excluding the intensive farm trap study (not conducted in 2006), almost twice as many ceratopogonids were captured in 2007 as compared to 2006. Hurricane Rita made landfall on September 24, 2005 as a category 3 hurricane near the Texas/Louisiana state line. St. Mary Parish received substantial wind and storm surge from this storm, which may have had an impact on larval habitat areas of *Culicoides* spp. and the subsequent 2006 population of adult ceratopogonids.

We did not capture any specimens of *C. insignis,* which is a coastal species known to transmit BTV-1 in the Caribbean. Kramer *et. al.* (1985) captured almost 60,000 specimens of *C. insignis* which accounted for over 99.9% of total midges captured in two years in Clewiston, Florida. Also, we did not catch any specimens of *C. sonorensis*, which is a very common species in the U.S. and is considered to be the primary vector of BTV. The larval habitat of *C. sonorensis* is muddy substrates with high organic matter, usually on the margin of a pond or stream (Blanton and Wirth 1979). An explanation for why we did not find *C. sonorensis* in our study could have been due to the low numbers of cattle (low organic matter) in the area.

The purpose of this study was to compare the efficacy of different trap types for capturing specimens of adult ceratopogonids in the coastal area of La. where a BTV-1 positive deer was found. The results of the study indicate that the miniature CDC black light trap baited with dry ice is highly effective for this purpose and should be included in future studies for capturing adult biting midges, especially specimens of *Culicoides* spp., on the coast of Louisiana.

CHAPTER 3. DETECTION OF BLUETONGUE VIRUS RNA IN FIELD-COLLECTED SPECIMENS OF *CULICOIDES* IN LOUISIANA

3.1 Introduction

Bluetongue virus (BTV), an *Orbivirus* in the family Reoviridae, is a double-stranded, segmented RNA virus known to infect many different domestic and wild ruminants. Bluetongue disease is classified as a notifiable multispecies disease by the Office of International Epizootics because of its substantial economic impact and potential for rapid spread. Infection with BTV can cause serious hemorrhagic disease with high mortality rates in sheep and deer (Osburn 1994; Howerth *et. al.* 1988). Other domestic ruminants, such as cattle and goats, rarely show clinical symptoms. Following infection of BTV, cattle have prolonged viremia in which BTV nucleic acid can be present in red blood cells for 16-20 weeks (MacLachlan *et. al.* 1994). Therefore, cattle can serve as reservoir hosts for BTV.

There are 24 described serotypes of BTV worldwide; BTV has been found where suitable vectors are present (Tabachnick 1996). Until recently, five serotypes (2, 10, 11, 13, and 17) of bluetongue virus were known to occur in the United States (Mullen *et. al.* 1999). In November 2004, bluetongue virus serotype 1 was isolated for the first time in the U.S. from a hunter-killed deer in the marsh area of the Atchafalaya Delta in St. Mary Parish, LA (Johnson *et. al.* 2006). Previously, the known range of BTV serotype 1 was confined to Central and South America (Tabachnick 1996).

The only known vectors of BTV are biting midges of the genus *Culicoides*, family Ceratopogonidae. In the New World, the primary vectors of BTV are considered to be *C. sonorensis* (Coquillett) in N. America and *C. insignis* (Lutz) in S. America and southern Florida (Mecham 2003; Tabachnick 2004; Blackwell 2004). The primary vector of bluetongue virus in the U.S. was considered to be *Culicoides variipennis* until Holbrook

et. al. (2000) divided *C. variipennis* into 3 different species: *C. sonorensis, C. variipennis,* and *C. occidentalis*.   Currently, the primary vector of BTV in the U.S. is considered to be *C. sonorensis* while *C. variipennis* and *C. occidentalis* are considered refractory to BTV infection (Tabachnick 2004).

Jochim *et. al.* (1966) first showed that BTV replicated in specimens of *C. variipennis* inoculated with BTV, and Bowne and Jones (1966) showed that BTV replicated in the salivary glands of *C. variipennis*. Kramer *et. al.* (1990) isolated BTV-11 using cell culture techniques from wild-caught *C. variipennis* in Colorado and Utah. Wieser-Schimpf *et. al.* (1993) reported BTV serotypes 2, 13, and 17 from seropositive cattle in Baton Rouge, La., and found 1 out of 135 pools of *C. variipennis* to be positive for BTV via PCR (the serotype was not reported).

Epizootic hemorrhagic virus (EHDV) and BTV are morphologically similar, but they differ antigenically. Like BTV, EHDV can affect all ruminants, but the disease is most severe in whitetail deer, or *Odocoileus virginianus* (Stallknecht and Howerth 2004). The clinical disease and symptoms in deer are identical for BTV and EHDV, and only virus isolations can differentiate the two viruses. At least 10 serotypes of EHDV are distributed worldwide (Gorman 1992); serotypes 1, 2, and 6 are found in the U.S (see Chapter 1, p. 15). Shope *et. al.* (1960) first isolated EHDV serotype 1 after a whitetail deer die off in 1955 in New Jersey.

Members of the genus *Culicoides* are the only known vectors of EHDV. In the U.S., the primary vector of EHDV is considered to be *C. sonorensis*. Jones *et. al.* (1977) isolated EHDV from parous specimens of *C. variipennis* (*C. sonorensis)* captured with modified CDC light traps in Kentucky. Foster *et. al.* (1977) showed that two different strains (NJ and KY) of EHDV could be transmitted by wild-caught *C.*

sonorensis; these two strains of EHDV were eventually named EHDV-1 (NJ) and EHDV-2 (KY). Furthermore, Foster *et. al.* (1980) isolated both strains of EHDV from cattle and wild-caught specimens of *C. sonorensis* during the same period of time. More recently, Smith *et. al.* (1996c) isolated EHDV-2 from specimens of *C. lahillei* (*C. debilipalpis*) and *C. variipennis* that had been fed on viremic deer and then tested 4-15 days later. In California, Rosenstock *et. al.* (2003) tested wild-caught specimens of *C. mohave* for EHDV using PCR, and reported that 35% of the pools were positive for EHDV.

Shortly after the first report of BTV-1 in St. Mary Parish, serum surveys were conducted on three nearby cattle farms which were located within 25 km of the area where the deer was shot. Two of these three farms had BTV-1 seropositive cattle. The purpose of this study was to identify potential BTV vectors in the area of apparent BTV-1 transmission. The objectives of the study were to collect ceratopogonids with light-traps at the three cattle farms, test the specimens for BTV RNA and EHDV RNA using IR-RT-PCR, and sequence any positive amplicons for serotype identification.

3.2 Material and Methods

▪ Farm Trap Study

From January 1, 2006, through November 15, 2007, trap studies were conducted at three cattle farms, two of which had BTV-1 positive cattle, in St. Mary Parish within 30 km of the location in the Atchafalaya Delta where the deer was shot (Chapter 2). Routinely, miniature CDC black light traps were hung from tree branches approximately 1.5-2.0 m above ground at each farm. There were two trap sites at each farm that were greater than 100 m apart; the traps were deployed twice per month. One trap at each farm was baited with 2 kg of dry ice in an igloo container. The dry ice was rotated between

the two sites at each farm every trap-night. Sealed, gelled-electrolyte six volt twenty amp hour rechargeable batteries (model 2.32; John W. Hock Co., Gainesville, FL) were used to power the traps and double ring fine mesh collection bags (model 1.45; John W. Hock Co., Gainesville, FL) were used to collect insects. The traps were set out before dusk and retrieved after sunrise. The nets were collected and immediately stored in a dry ice container with approximately 15 kg of dry ice. The nets were transported to LSU and stored at -80 ° C.

Nets were emptied into a large Petri dish and insects were sorted on a chill table (BioQuip®, Gardena, CA) using a dissecting microscope. All ceratopogonids were separated into genus, and the specimens in the genus *Culicoides* were furthered sorted by species. Specimens were sorted by examining the wing veination, number of antennal segments, spermathecae, and maxillary palps using keys in the Manual of Nearctic Diptera (1981) and of Blanton and Wirth (1979). Specimens were separated by species, site, and date and placed into labeled 1.5ml vials chilled on dry ice. Pools of flies were created by separating ceratopogonids by species, farm, and month captured and placing them into chilled vials; pools contained a minimum of 1 specimen and a maximum of 50 specimens. The vials were then stored at -80 ° C. Subsequently, the vials were packaged in a styrofoam ice chest containing dry ice and transported to the Arthropod-Borne Animal Diseases Research Laboratory (ADABRL) in Laramie, WY, to be screened for bluetongue virus.

▪ Intensive Trap Study

An intensive trap study trial was conducted at farms A and C from May 22- June 6, 2007, and a second trial was conducted at farms A and B from August 21- September 3, 2007 (Chapter 2). Three traps types were used: 1) New Jersey Stainless Steel Light

Trap with a 40W incandescent light bulb (model 1112; John W. Hock Co., Gainesville, FL), 2) New Jersey Stainless Steel Light Trap modified with black light, and 3) miniature CDC black light trap baited with 2kg of dry ice. The New Jersey trap was modified with a black light according to Wieser-Schimpf *et. al.* (1991) except we used a 120V 60Hz ballast (Lot # LQ206FTP; Advance) and two F8T5 8W black light bulbs. A rechargeable sealed AGM twelve volt 100 amp hour battery and a 600 watt DC to AC power inverter was used to power the New Jersey Traps where 110 V AC was not available. The twelve volt battery and power inverter were placed in a dry, sealed toolbox.

All traps were hung from a tree branch at approximately 1.5-2.0 m above the ground and double ring, fine mesh collection bags were used on all traps. The traps were rotated daily three times a week for three consecutive weeks for 27 trap nights per farm for a total of 54 trap nights for each of the two trials. Nets were collected after sunrise and stored in a dry ice container until being transported back to LSU campus. The nets were stored at -80 º C and insects were processed by the methods described above. These ceratopogonids also were transported to ADABRL in Laramie, WY and screened for BTV.

▪ Infrared Reverse Transcriptase Polymerase Chain Reaction

At ABADRL, the vials of adult ceratopogonids were stored in at -80 º C until the infrared reverse transcriptase PCR (IR-RT-PCR) assay was performed. The pools of female flies were macerated separately in gnat homogenization buffer (GAM) with gold plated tungsten beads (Spirit River, Roseburg, OR) using a Tissue Lyser (Qiagen, Valencia, CA) as previously described by Kato and Mayer (2007). Total RNA was extracted from the homogenate using an RNeasy kit (Qiagen) following the manufacturers recommendations. Gnat homogenization buffer consisted of 400 µg/ml

penicillin, 400 µg/ml streptomycin, 200 µg/ml gentamicin, 25 µg/ml ciprofloxacin, and 5 µg/ml amphotericin B prepared in Medium 199 with Earle's salts (M199-E) in 10% fetal bovine serum. The extracted RNA was screened by IR-RT-PCR for BTV and EHDV using the protocols described by Kato and Mayer (2007). Infrared labeled primers EHDV 63-F1 (5'-AACAGTTACTACGCAAATCA-3') and EHDV 245-R1 (5'-AGCCA TTTCAGCCAATCT-3') were used to amplify a portion of the NS1 gene of EHDV and BTV specific primers BTV 12F (5'-TCGCTGCCATGCTATCCG-3') and BTV 246R (5'-CGTACGATGCGAATGCAG-3') were used to amplify the highly conserved regions of the S10 gene of BTV.

We also attempted to isolate virus from positive pools. An inoculum of 100 µl of homogenate and GAM was added to 100 µl of Vero cells (2.5×10^5 cells/ml) with M199-E in triplicate. The cells were incubated at $37\,^{\circ}$ C with 5-6% CO_2 for 7 days. Cells were checked for cytopathic effect (CPE) after 48 hours and 7 days. After 7 days, the cells were disrupted and passed onto fresh Vero cells without GAM and cells were checked for CPE.

The amplicons (PCR products) of positive pools were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California) and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) using PCR primers. Sequences were determined using an ABI 3700 capillary sequencer (Applied Biosystems, Foster City, California), aligned and assembled with Chromas Lite 2.01 (Technelysium, Australia) and ClustalW (Kyoto University Bioinformatics Center, Japan), and compared to sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, Maryland).

3.3 Results

A total of 2,309 specimens (264 pools) of ceratopogonids representing 10 species of *Culicoides* and two other genera (*Atrichopogon* spp. and *Forcipomyia* spp.) of ceratopogonids were screened for BTV using IR-RT-PCR (Table 1). Of these pools, five tested positive for the presence of BTV. All specimens of positive pools were captured using miniature CDC black light traps. There was at least one positive pool from each of the three locations. The amplicons of the positive pools were sequenced for serotype. We sequenced NS3 gene from RNA extracts, which were 100% identical to that of BTV-13 or BTV-17 (AY426597.1, AF044713.1, AF044712.1, AF044375.1, AF044374.1, L08630.1). None of the amplicons were matches for BTV serotype 1.

There were 80 pools of 681 specimens from farm A. Out of nineteen pools of *C. debilipalpis*, one was positive for BTV and these specimens were captured in October 2006. One pool of a single specimen of *C. haematopotus* was positive for BTV; the specimen was captured in August 2007. A total of 820 specimens in 67 pools from Farm B were screened for BTV. One out of 11 pools of *C. crepuscularis*, captured in June 2006, and 1 out of 13 pools of *C. debilipalpis*, captured in September 2006, were positive for BTV. A total of 117 pools of 808 specimens from Farm C were screened for BTV, and one of 3 pools of *C. furens* was positive for BTV. The positive pool was a single specimen of *C. furens* that was captured in May 2006. There were no positive pools for EHDV and no virus grew on cell culture.

3.4 Discussion

Specimens of four different ceratopogonid species (*C. crepuscularis, C. debilipalpis, C. haematopotus,* and *C. furens*) were found to be positive for BTV serotype 17 or 13. Three of these species (*C. crepuscularis, C. haematopotus,* and *C. furens*) have

Table 3. 1. Number of pools and specimens of ceratopogonids captured at farms in 2006 and 2007 in St. Mary Parish, La. and tested for BTV and EHDV by IR-RT-PCR.

Location	Species/Genus	# Pools	# Specimens
Farm A	*C. arboricola*	23	229
	Atrichopogon spp.	2	10
	C. biggutatus	1	2
	C. crepuscularis	16	56
	*C. debilipalpis**	19	201
	Forcipomyia spp.	16	217
	C. furens	1	1
	*C. haematopotus**	14	36
	C. hinmani	8	16
	C. paraensis	11	32
	C. stellifer	6	8
Farm B	*C. arboricola*	11	204
	Atrichopogon spp.	5	13
	C. biggutatus	1	1
	*C. crepuscularis**	11	89
	*C. debilipalpis**	13	245
	Forcipomyia spp.	18	241
	C. haematopotus	1	1
	C. hinmani	1	13
	C. paraensis	4	8
	C. stellifer	2	5
Farm C	*C. arboricola*	13	120
	Atrichopogon spp.	9	79
	C. biggutatus	2	3
	C. crepuscularis	13	105
	C. debilipalpis	13	180
	Forcipomyia spp.	10	129
	*C. furens**	3	17
	C. haematopotus	11	32
	C. hinmani	1	1
	C. paraensis	3	12
	C. stellifer	2	3
TOTAL		264	2309

* denotes one BTV positive pool

not been highly considered as vectors of BTV in the U.S..

Previous studies have incriminated *C. debilipalpis* as a vector a BTV. This species is known to feed in large numbers on deer, especially in the southern U.S. One study in Georgia reported capturing over 20,000 specimens of *C. debilipalpis* from a caged deer in one morning (Smith *et. al.* 1996a). Moreover, Mullen *et. al.* (1985) showed that laboratory-fed *C. debilipalpis* were able to harbor BTV through replication and transmit the virus 14 days after a blood meal. Also, Smith *et. al.* (1996) isolated EHDV-2 from specimens of *C. lahillei* (*C. debilipalpis*) that had been fed on viremic deer and then tested 4-15 days later.

Female specimens of *C. crepuscularis* are considered to be ornithophillic, but specimens have been reported biting man and feeding on ewes and steers (Pickard and Snow 1955; Reich *et. al.* 1997). This species was dominant in the marsh area where the BTV-1 positive deer was shot, composing of over 95% of the total specimens of *Culicoides* captured (see Chapter 2). In northern Colorado, White *et. al.* (2005) found genome segments 7 and 3 of BTV in larvae of *C. crepuscularis* collected in cattle pastures, but BTV was not isolated from the larvae.

Specimens of *C. furens* are known to feed on man and are very abundant in coastal areas. In Florida, this species is the most important human-feeding midge (Linley 1983). Some specimens were also captured in the light-trap where the BTV-1 positive deer was shot (see Chapter 2). This species has been termed as a potential vector of BTV in Central America and the Caribbean region (Saenz *et. al.* 1994).

Specimens of *C. haematopotus* are largely found associated with livestock and wooded areas throughout the U.S. (Blanton and Wirth 1979). Mullen *et. al.* (1999) captured some specimens of *C. haematopotus* off of cattle in Alabama while conducting

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an experiment to find potential vectors of BTV. Smith *et. al.* (1996b) reported capturing

some specimens in Georgia while surveying an area with an enzootic of BTV and EHDV.

Khalaf (1969) captured some specimens in light-traps in Louisiana from April until

October. However, no previous studies in the U.S. have shown any positive field-

collected specimens of *C. haematopotus* for BTV.

We did not catch any specimens of *C. sonorensis*, which is the main vector of

BTV in the U.S. Nor did we capture any specimens of *C. insignis*, which is the vector of

BTV-1 in the Caribbean and Central and South America. During 2005, the Southeastern

Cooperative Wildlife Disease Study group collected and tested serum samples from 399

hunter-killed deer in La. and found 6 deer to be antibody positive for BTV-1. Three of

the six deer came from St. Mary Parish which indicates that BTV-1 is being transmitted

in the area of our study (Stallnecht 2006). Therefore, we can assume that BTV-1,

regardless of its introduction the U.S., can be transmitted by native species of *Culicoides*,

other than *C. sonorensis*.

In La., bluetongue disease in deer normally occurs in the fall months of the year

(Enright, F. personal communication). Vertical transmission of BTV in insects has not

been proven and BTV is not contagious. Thus, an insect vector must take a blood meal

from an animal infected with BTV, and the virus has to replicate in the salivary glands of

the insect before it can be transmitted (approximately 14 days). Death in deer occurs

approximately 14 days after infection. Therefore, it takes approximately one month for an

insect vector of bluetongue virus to transmit the virus to a healthy deer and for that

animal to die. Therefore, species of *Culicoides* that are abundant right before and during

the fall would be suspect vectors. In our study, BTV-positive specimens of *C.

debilipalpis* were captured in September and October (Figure 3. 1); there was a large

population peak of this species in August and September, and some specimens were caught in October. Since we collected BTV positive specimens of *C. debilipalpis* during the time when BTV outbreaks in deer occur, and Mullen *et. al.* (1985) showed that *C. debilipalpis* was a competent BTV vector, this species should be highly considered as a potential BTV vector in south La.

We did not capture any BTV-1 or EHDV positive ceratopogonids. Potentially capturing and testing more ceratopogonids in the area would be adequate for finding BTV-1 positive specimens. On the other hand, BTV-1 could be transmitted by insects that are not captured in light-traps or even insects that were captured but not examined for BTV, such as mosquitoes. More studies are needed to characterize the epidemiology of BTV serotype 1 and EHDV in south Louisiana.
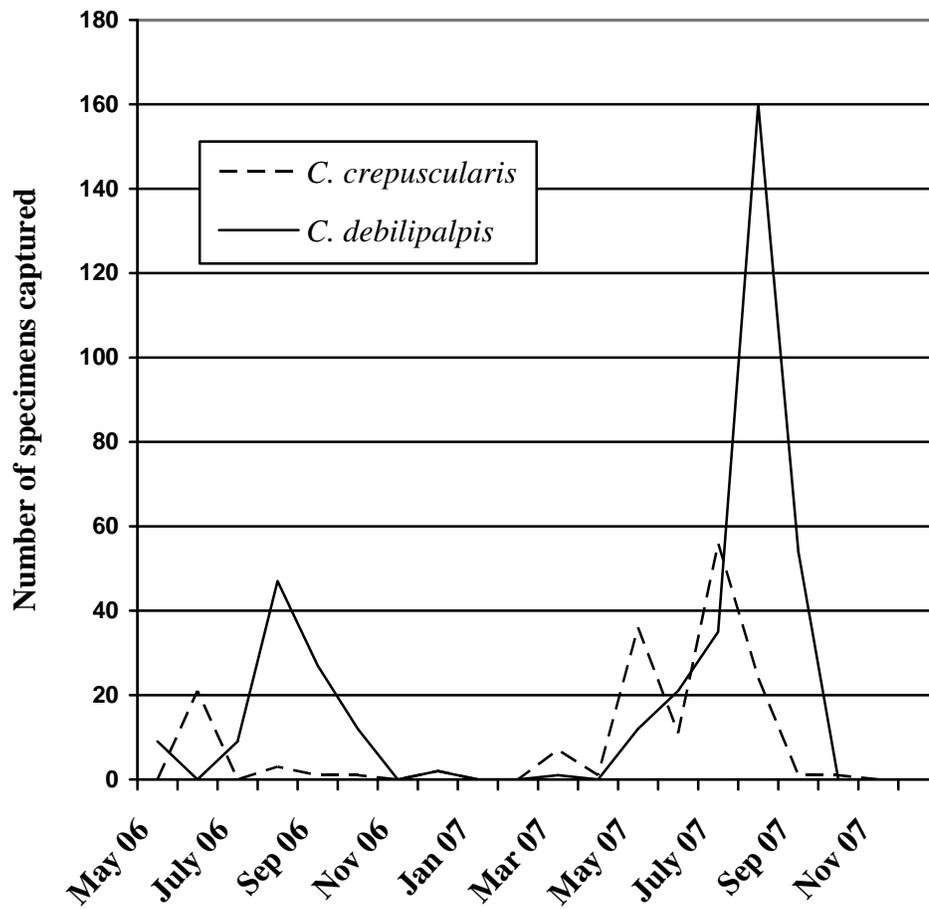
Figure 3. 1. Total number of specimens of *C. crepuscularis* and *C. debilipalpis* caught in CDC light-traps baited with dry ice on three farms in St. Mary Parish, La.

SUMMARY AND CONCLUSIONS

In November 2004, bluetongue virus (BTV) serotype 1 was detected for the first time in the U.S. in a hunter-killed deer in the marsh area of the Atchafalaya Delta in St. Mary Parish, LA (Johnson *et. al.* 2006). Subsequent serum surveys on three cattle farms further inland from the area where the deer was shot found bluetongue virus serotype 1 positive cattle on two of the three farms. The only known vectors of BTV are biting midges of the genus *Culicoides*, family Ceratopogonidae. The primary vector of BTV-1 in Central and South America is considered to be *C. insignis*, while the primary vector of BTV in the U.S. is *C. sonorensis.*

Seven sites were established in the immediate area (marsh) where the deer was found from January 2006 to November 2007 and miniature CDC light traps were deployed once per month at each site. Also, two CDC light traps were deployed twice per month at each of the three cattle farms. Dry ice was used as bait for one CDC trap at each farm and the dry ice was rotated between the two traps each trap-night. In 2007, an intensive trap study was conducted twice at two of the three farms where three different types of traps were run for three consecutive nights per week for three weeks in a row. The trap types were a New Jersey trap with an incandescent bulb, a New Jersey trap with a black light bulb, and a CDC trap baited with dry ice.

Specimens of 10 different species of *Culicoides* were captured at the farms and specimens of 7 of these 10 species were caught in the Atchafalaya Delta marsh area. In the entire study, 8,179 ceratopogonids were captured including 5,068 in the genus *Culicoides*. CDC light traps baited with dry ice caught significantly more flies than traps without dry ice for three species which accounted for over 80 percent of the *Culicoides* spp. captured: *C. arboricola, C. debilipalpis,* and *C. crepuscularis*. CDC light traps with

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dry ice also had significantly higher catches than the New Jersey light traps for *C. arboricola, C. debilipalpis,* and *C. crepuscularis*.

Infrared reverse transcriptase PCR was performed to screen for BTV and EHDV on 264 pools representing 2,309 specimens. All positive samples were sequenced for serotype determination. Five pools out of 275 were positive for BTV. Pools of four species of *Culicoides* were found to be positive: *C. crepuscularis, C. debilipalpis* (2 pools), *C. haematopotus,* and *C. furens*. The amplicons of the positive specimens were sequenced and found to be identical to either BTV-17 or BTV-13.

The results of the study indicate that the miniature CDC black light trap baited with dry ice is highly effective for capturing high numbers of adult biting midges (especially *Culicoides* spp.) on the coast of La. and should be included in future studies in this area. Also, BTV-positive specimens of *C. debilipalpis* were captured in September and October, and there was a large population peak of this species in August and September. Since we collected BTV positive specimens of *C. debilipalpis* during the time when BTV outbreaks in deer occur in La., and Mullen *et. al.* (1985) showed that *C. debilipalpis* was a competent BTV vector, this species should be highly considered as a potential BTV vector in south La.

We did not capture any BTV-1 or EHDV positive ceratopogonids. Potentially capturing and testing more ceratopogonids in the area would be adequate for finding BTV-1 or EHDV positive specimens. On the other hand, BTV-1 or EHDV could be transmitted by insects that are not captured in light-traps or even insects that were captured but not examined for BTV, such as mosquitoes. More studies are needed to characterize the epidemiology of BTV serotype 1 and EHDV in south Louisiana.

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APPENDIX : MAP OF SITES SAMPLED FOR CERATOPOGONIDS IN ST. MARY PARISH, LOUISIANA
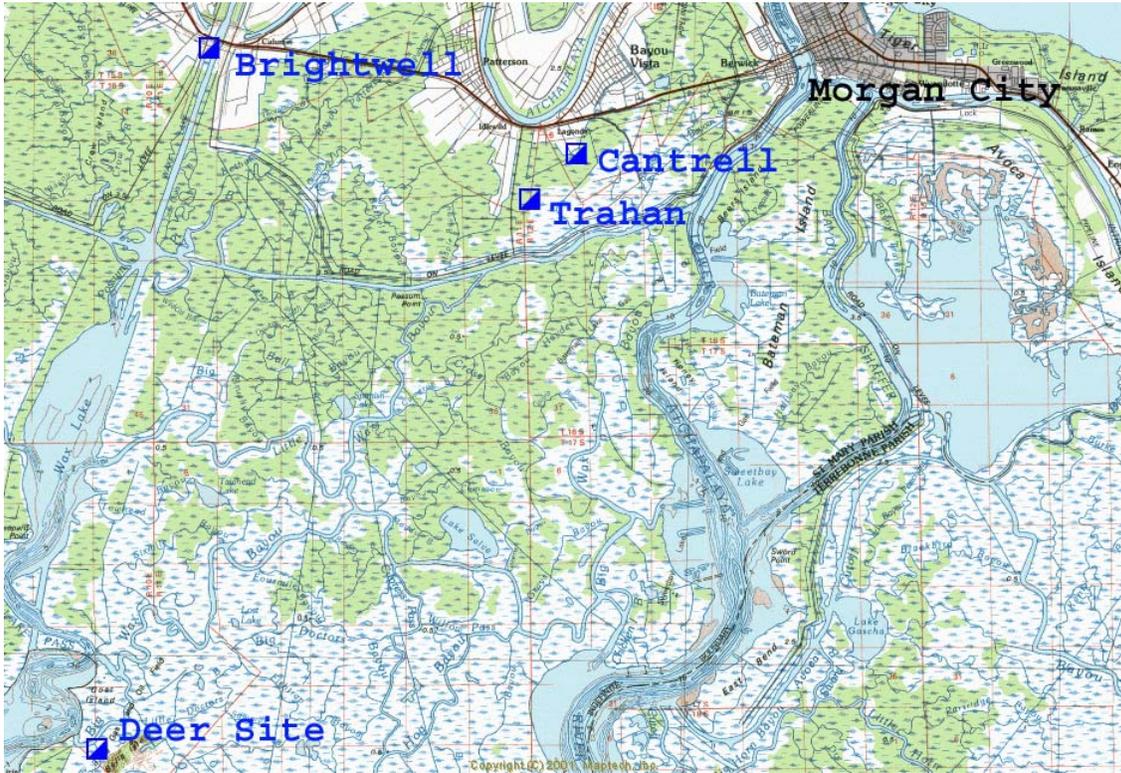
Key: Cantrell=Farm A, Trahan=Farm B, Brightwell= Farm C, Deer Site=Atchafalaya Delta Marsh.

VITA

Michael Edward Becker was born to Edward and Kathleen Becker on April 2, 1982 in New Orleans, Louisiana. He received his bachelor of science degree majoring in biological sciences at Louisiana State University in December of 2004. In his last semester before graduation, he was employed as a student worker by Cole Younger in the Department of Entomology at LSU. Upon graduation, he became a transient worker under Lane Foil and worked until January 2006 when he became a graduate student in the Department of Entomology at LSU. He was then employed as a graduate assistant under Lane Foil working on a bluetongue virus project in south Louisiana. Michael received his master of science in entomology in August 2008.