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# *CULICOIDES VARIIPENNIS* AND BLUETONGUE-VIRUS EPIDEMIOLOGY IN THE UNITED STATES[1]

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ABSTRACT

The bluetongue viruses are transmitted to ruminants in North America by *Culicoides variipennis*. US annual losses of approximately $125 million are due to restrictions on the movement of livestock and germplasm to bluetongue-free countries. Bluetongue is the most economically important arthropod-borne animal disease in the United States. Bluetongue is absent in the northeastern United States because of the inefficient vector ability there of *C. variipennis* for bluetongue. The vector of bluetongue virus elsewhere in the United States is *C. variipennis sonorensis*. The three *C. variipennis* subspecies differ in vector competence for bluetongue virus in the laboratory. Understanding *C. variipennis* genetic variation controlling bluetongue transmission will help identify geographic regions at risk for bluetongue and provide opportunities to prevent virus transmission. Information on *C. variipennis* and bluetongue epidemiology will improve trade and provide information to protect US livestock from domestic and foreign arthropod-borne pathogens.

## INTRODUCTION

Arthropod-borne pathogens cause significant mortality and morbidity to humans and animals. The bluetongue viruses, which cause bluetongue disease in ruminants, are among the most important arthropod-borne animal pathogens in the United States. The primary North American arthropod vector of the bluetongue viruses is a biting midge, *Culicoides variipennis* (Diptera: Cera-

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topogonidae). International regulations prohibit the movement of livestock and their germplasm from countries harboring animals with bluetongue viruses to countries with livestock considered virus free. Many US livestock populations are infected with bluetongue viruses or are located in areas endemic for the disease. Consequently, the US livestock industry has suffered estimated annual losses of $125 million because of lost trade in cattle, sheep, or their germplasm to bluetongue-free countries, such as those in the European Union (24, 88).

This paper focuses on the role of *C. variipennis* in bluetongue disease epidemiology. We must understand the mechanisms controlling *C. variipennis'* ability to vector the bluetongue viruses if we are to reduce the effects of bluetongue disease in North America. Investigations of other arthropod-borne, disease-causing pathogens involve the same issues discussed here: (*a*) the importance of the vector in disease epidemiology, (*b*) the importance of the vector in pathogen biology, and (*c*) features of the vector that provide opportunities for controlling the disease.

## BLUETONGUE DISEASE

Bluetongue disease was first described in 1902 as malarial catarrhal fever in South African sheep (39). Soon afterwards, the disease agent was recognized as filterable (109). In 1944, South African species in the genus *Culicoides* were recognized as vectors of bluetongue virus (15). From their origins in Africa, bluetongue viruses have spread to the Middle East, Asia, the Americas, and Australia (25). Bluetongue disease in the United States was first described as "soremuzzle" in Texas (33), and bluetongue virus was isolated from sheep with soremuzzle in California in 1952 (64). *C. variipennis* was subsequently identified as a vector in the United States (94).

### *Bluetongue Pathogenesis*

Several reviews discuss bluetongue-virus pathogenesis in ruminants (62, 89, 90). Bluetongue viruses can infect several domestic and wild ruminant species, but the most significant diseases occur in sheep. Clinical signs include a rise in temperature lasting 5–7 days; hyperemia and swelling of the buccal and nasal mucosa; profuse salivation; swollen tongue; hemorrhage in the mucosal membranes of the mouth; oral erosions; and hemorrhage in the coronary bands of the hoof, which produces lameness. Sheep may vomit because of lesions in the esophagus and pharynx, which can lead to aspiration of the ruminal contents, pneumonia, and frequently death. The severity of the disease varies according to virus serotype and is less drastic in indigenous than in introduced sheep (18, 40, 89, 90, 128). Although sheep mortality may range from 5 to

50%, bluetongue-virus infections in many regions of the world produce no overt disease (90).

Clinical bluetongue disease in cattle is rare. Cattle develop a prolonged viremia lasting several weeks, which may allow the virus to survive during winter or other times when vector populations are absent or small. Controversy has surrounded the extent of clinical signs. Currently, cattle are thought to develop signs of bluetongue disease rarely («5% of infected animals), and the virus is considered to have little if any effect on reproduction. However, early prenatal infection may lead to embryonic death. Fetuses infected at later stages of gestation survive without persistent infection, and infected animals develop specific antibodies (62, 89–92).

## *Bluetongue Viruses*

Bluetongue viruses are double-stranded RNA viruses in the genus *Orbivirus,* family Reoviridae. They have 24 serotypes worldwide. The bluetongue genome (molecular weight $12 \times 10^6$) can be resolved via polyacrylamide electrophoresis into 10 gene segments, which encode ten mRNAs for seven structural and three nonstructural proteins. The viruses are icosahedral particles with the RNA genome encapsidated in a double-layered protein coat (38, 59, 101, 102). The outer coat contains two major proteins, VP2 and VP5, while the inner coat consists of two major proteins, VP3 and VP7. Serotype specificity resides in VP2. VP7 contains epitopes that react with group-specific bluetongue antibodies. The roles of the minor core proteins (VP1, VP4, and VP6), as well as of the nonstructural proteins (NS1, NS2, NS3), have been described (17).

The bluetongue-virus proteins function differently in mammalian and insect cells. Treatment of viruses with trypsin or chymotrypsin results in cleavage of VP2 from the outer capsid, producing an infectious subparticle. Further treatment uncoats the inner core (71). Although inner-core particles are not as infectious as intact virus to mammalian cells, all three particles are equally infective to susceptible *C. variipennis.* (68).

The genetic diversity among bluetongue serotypes and related orbiviruses, e.g. African horse sickness viruses and epizootic hemorrhagic disease viruses, is known (28–30, 100–102). Nucleotide sequences for VP3, VP5, VP7, NS1, and NS3 reveal close genetic relationships between orbiviruses from the same geographic region (29, 30). Bluetongue viruses from Australia form a distinct topotype. Within each region, topotypes contain similar serotypes. For instance, VP3 sequences show that BLU-1 in Australia is related to the Australian topotype consisting of serotypes 3, 9, 16, 20, 21, and 23, whereas BLU-1 in South Africa is closer to South African serotypes 3 and 9 (29). A close phylogenetic relationship, based on VP3 gene-sequence data, between a US

BLU-2 isolate and serotypes 1, 6, and 12 from Jamaica and Honduras supports proposals that BLU-2 was introduced into the United States from the Caribbean (95, 106).

The relationships between viral diversity and the different *Culicoides* vectors present in different regions are unknown. Studies of an RNA arbovirus, vesicular stomatitis virus, suggested that arboviruses evolved in a punctuated fashion as they entered new environments and were transmitted by new arthropod vectors (84). New variants of bluetongue virus could also result from gene segment reassortment between serotypes. Reassortment frequencies are higher in *Culicoides* vectors than in sheep (103). The influence of reassortment on the population biology of the bluetongue viruses is unknown (27). However, *Culicoides* vectors clearly influence bluetongue-virus variation and biology (123).

## *Bluetongue Epidemiology*

WORLDWIDE Bluetongue viruses are distributed worldwide between latitudes 40°N and 35°S. They infect ruminants wherever suitable *Culicoides* vectors are present. There are regional differences in the viruses, in species of *Culicoides,* and in clinical signs in infected animals. Clinical bluetongue disease is not generally seen in the Central American–Caribbean Basin, where BLU-1, -3, -4, -6, -8, -12, -14, and -17 have been observed, presumably vectored by *C. insignis* (124, 127). Similarly clinical disease is not generally observed in Australia, where BLU-1, -3, -9, -15, -16, -20, -21, and -23 are transmitted by *C. wadai, C. brevitarsis, C. fulvus,* and *C. actoni* (110, 111). In Asia, various *Culicoides* spp. are the suspected vectors for BLU-1, -2, -3, -9, -12, -14–19, -20, -21, and -23 (34, 112, 126). Clinical disease does appear in Africa and the Middle East, where serotypes 1–19, 22, and 24 are found. There, the primary vector is *C. imicola,* although *C. tororoensis, C. milnei, C. obsoletus,* and *C. schultzei* may play minor roles (68). In many regions of the world, the vectors of the bluetongue viruses are unknown (123).

Bluetongue viruses caused disease outbreaks between 1956 and 1960 in Spain and Portugal (97). The principal vector, *C. imicola,* was also the vector of the related African horse sickness viruses in Spain (70). The potential for bluetongue virus in Europe has resulted in animal health restrictions to ensure bluetongue-free animal imports. The range of *C. imicola* in Europe does not extend beyond the Iberian Peninsula because of inhospitable climate (97). However, *C. obsoletus* and *C. pulicaris,* capable vectors of bluetongue in the laboratory (43), are common in Northern Europe (69). Without an understanding of the vector ability of European *Culicoides* spp. in the field, bluetongue incursions into Europe remain a concern.

NORTH AMERICA US bluetongue serotypes are 2, 10, 11, 13, and 17 (3, 26). In a serologic survey for bluetongue-virus antibody in US cattle, Metcalf et al (72) examined ~20,000 sera and found that 17.8% were positive for bluetongue antibody. Bluetongue antibody prevalence ranged from 0 to 79% in different states. It was highest in southern and western states and lowest in northern states, where prevalence was ≤2%. These values were confirmed in several serologic surveys conducted during the ensuing two decades involving thousands of additional samples of cattle sera (93). Seropositive animals have been traced to origin to determine the contribution of animal movement to the presence of bluetongue-positive animals in northern states. Of more than 32,000 cattle tested in New York State, only 14 were seropositive, and all of these were originally from high seroprevalence regions of the United States (58).

Bluetongue viruses were found in the Okanagan Valley of British Columbia in 1976 and 1987 (113). The dire implications for the Canadian livestock trade prompted a serosurvey of more than 175,000 cattle between 1976 and 1992 (14, 113). BLU-11 was identified (13), but virus was not observed in collections of *C. variipennis* in the Okanagan Valley (65), and evidence of bluetongue-virus infection outside the valley has not been observed (14). Bluetongue viruses may have been transmitted to animals in this region after being introduced from the United States. However, despite this example of sporadic transmission, these viruses are apparently not endemic to Canada (14).

## *CULICOIDES VARIIPENNIS*

Much evidence suggests that *C. variipennis* is the primary North American vector of the bluetongue viruses: (*a*) The species is widespread. (*b*) Many studies show it feeds on wild and domestic ruminants. (*c*) In the laboratory, feeding on susceptible ruminant hosts has resulted in infection, and under laboratory conditions, it transmits virus to susceptible hosts. (*d*) Bluetongue viruses have been isolated on numerous occasions from field-collected *C. variipennis* (2, 8, 10, 20–23, 41, 46, 48, 50, 51, 60, 61, 63, 66, 67, 82, 85, 86, 103, 108, 114, 116, 122, 125, 131, 134). *C. insignis* vectors the bluetongue viruses in South and Central America and through the northern extension of its range in southern Florida (31, 55, 124). Little evidence points to a major role for other *Culicoides* species in North America. *C. venustus* (53), *C. debilipalpis,* and *C. stellifer* (74) support little or no infection in the laboratory, and other species have not been incriminated in bluetongue epizootics. *C. brookmani* or *C. boydi* might serve as bluetongue vectors for desert bighorn sheep in areas of California, as indicated by their abundance and the near absence of *C. variipennis* in these habitats (78). Various models attempting to

predict bluetongue-virus transmission are based on climactic variables relating to insect activity (44, 135).

## *Systematics*

*C. variipennis* is in the subgenus *Monoculicoides.* Based on morphologic variations in collections throughout the United States, *C. variipennis* was divided into five subspecies: *C. variipennis variipennis, C. variipennis sonorensis, C. variipennis occidentalis, C. variipennis australis,* and *C. variipennis albertensis* (132). Other workers believed these forms were species because of the absence of morphologic hybrids in regions where they were sympatric (12, 54). However, studies with laboratory-reared *C. variipennis* suggested that some of the morphologic characters were modified by the environment and thus invalid for use in classification (35). The difficulty in defining subspecies relationships resulted in a single grouping known as the *C. variipennis* complex (133).

Isozyme electrophoretic analyses of ~200 *C. variipennis* populations from the United States have helped define population and subspecies genetic relationships (36, 37, 115, 118). Populations, analyzed for genetic variation using 11–21 different isozyme-encoding loci, confirmed only three members in the *C. variipennis* complex (36, 37, 118): *C. variipennis variipennis* (northern regions of the United States), *C. variipennis sonorensis* (from Florida to California; north to Virginia and Ohio; and in the west as far north as Washington and British Columbia), and *C. variipennis occidentalis* (Arizona to California, north to Washington and British Columbia).

Limited gene flow was found between California *C. variipennis sonorensis* and *C. variipennis occidentalis* populations (36, 118). *C. variipennis sonorensis* larvae resided in highly polluted organic habitats, while *C. variipennis occidentalis* larvae inhabited highly saline habitats, e.g. Borax Lake, California (36). Collections from approximately 100 New England sites yielded only *C. variipennis variipennis* populations (37). No isozyme genes diagnostic for subspecies have been identified, although other molecular markers can be used (96). However, gene frequencies, and genetic similarities based on gene-frequency differences, showed that populations within a subspecies are more closely related to each other than to populations from other subspecies, regardless of geographic proximity. Populations classified morphologically as *C. variipennis australis* are genetically *C. variipennis sonorensis* (FR Holbrook & WJ Tabachnick, unpublished observations). Populations from the Gulf Coast of the United States in which *C. variipennis variipennis* and *C. variipennis sonorensis* occurred in the same larval habitat lacked any genetic hybrids (FR Holbrook & WJ Tabachnick, unpublished observations). This observation suggests that the three subspecies are indeed separate species. However, pending

formal descriptions, they should continue to be referred to using the subspecies designation.

## *Population Genetics*

Active *C. variipennis sonorensis* adults are not present during the winter in Colorado. Populations overwinter predominantly as larvae in permanent aquatic habitats (5, 6). In one study in this region (117), the gene frequencies of permanent populations remained stable through two seasons at all but two loci. *C. variipennis sonorensis* populations collected from temporary larval sites, which did not persist through the winter, showed genetic changes each summer that resulted from chance effects when these habitats were colonized each spring (117). Migration, at a rate of ~2.15 *C. variipennis sonorensis* per generation (regardless of population size), allowed temporary populations to differentiate from permanent populations through chance and prevented permanent populations from differentiating from one another. This study defined the major features of Colorado *C. variipennis sonorensis* population genetics (117): (*a*) Permanent larval populations maintain genetic stability; (b) no migration occurs between permanent populations during the winter; and (*c*) temporary populations are founded each spring and differ from permanent populations owing to chance (117). Although *C. variipennis* may disperse several kilometers (56; FR Holbrook, personal communication), as well as longer distances via wind (105–107), such dispersal did not affect population differentiation. Temporary populations, separated by only a few hundred meters, were not panmictic and were genetically differentiated (117). Weather is the major factor shaping the genetic structure of Colorado *C. variipennis sonorensis* populations.

Differentiation among other US populations provides additional evidence for the effect of weather on *C. variipennis* population dynamics and genetic structure. One measure of genetic variation between two populations is the average (av) genetic distance (*D*) based on allele-frequency differences. The av *D* among all populations in a region compared with the av *D* in another region quantifies the differences in regional genetic diversity. The av $D \pm$ SE ($n$ = number of pairwise population comparisons) showed significant differences (36, 37): among New England *C. variipennis variipennis* populations, av $D = 0.046 \pm 0.002$ ($n = 276$); among Colorado *C. variipennis sonorensis,* av $D = 0.040 \pm 0.010$ ($n = 21$); among California *C. variipennis sonorensis,* av $D = 0.010 \pm 0.007$ ($n = 171$); and between California *C. variipennis occidentalis,* av $D = 0.132 \pm 0.017$ ($n = 10$). *C. variipennis sonorensis* populations within a single Colorado county exhibited levels of genetic diversity similar to those of New England *C. variipennis variipennis.* Both of these populations have significantly higher genetic diversity than California *C. vari-*

*ipennis sonorensis* collected throughout the state. In Colorado and New England, temporary populations arising each spring probably generate temporally differentiated populations and thus greater genetic diversity. Populations of *C. variipennis sonorensis* in California, which enjoy longer seasons and have active adult migration, experience greater gene flow and less genetic differentiation (36). In contrast, California *C. variipennis occidentalis* populations showed the highest genetic differentiation, as a result of their wide geographic separation from one another and a lack of gene flow with nearby *C. variipennis sonorensis* (36).

In summary, the three major groups in the *C. variipennis* complex share only limited gene flow; their population genetics are influenced primarily by weather; and their distributions are associated with the North American distribution of the bluetongue viruses. The distribution of bluetongue in the United States has been stable for more than 20 years, despite potential change resulting from animal movement within the United States and into Canada, and from migration of infected *C. variipennis* between regions. However, infected exotic *Culicoides* spp. could still enter the United States (105), as could foreign livestock carrying exotic forms of the bluetongue viruses, particularly those from the Central American–Caribbean Basin. *Culicoides* spp. do not respect national, regional, or political boundaries (69).

## *Vector Capacity*

Traits associated with arthropod ability to transmit pathogens, such as host preference, biting or feeding rates, gonotrophic cycle, population densities, and vector longevity, determine vector capacity. Vector capacity also depends on vector competence, which involves the ability of the vector to be infected with the pathogen, the ability of the vector to infect progeny by transovarial transmission, and the ability of the vector to transmit the pathogen to a suitable host (see 9, 11, 32, 73, 98, 120).

Information on *C. variipennis* vector capacity is limited. Population densities vary throughout geographic regions. Although *C. variipennis variipennis* larvae and adults are often found on dairies throughout New York State (80, 104), *C. variipennis sonorensis* larvae and adults on southern California dairies are even more abundant—by one to two orders of magnitude (75, 76). If biting rates are related to adult density, this relationship would explain why bluetongue viruses are not endemic in the northeastern United States, where biting rates are probably substantially reduced (77). Flight activity may also influence biting rates. *C. variipennis* flight activity depends on light intensity and temperature, and most flight occurs at dusk and dawn (6, 57). Population variation regarding flight has not been studied.

Another component of vector capacity is the daily survivorship of adult

females. Infected females must survive the incubation period to allow the pathogen to replicate so that transmission to an animal may follow. The extrinsic incubation period is 10–14 days at 23°C in *C. variipennis* (21) but varies substantially with temperature (82). Survivorship estimates of *C. variipennis* in the field are based on parity rates, determined by observing pigment granules deposited in the abdominal cuticle after blood feeding (1, 16), and on estimates of the gonotrophic cycle determined by examining degenerative relics in the ovariole pedicel (5, 81). The daily survivor rate in New York State was estimated at 0.62–0.88 (80), and a similar survivor rate was estimated in the western United States (83, 134). Since these data are based on estimates of gonotrophic cycles that are governed by temperature (79), more field studies are needed to assess population variation.

The limited information on vector capacity indicates population and regional variations in *C. variipennis* biting rates, extrinsic incubation time, and perhaps—although more data are needed—daily survivorship. Clearly more results must be gathered about variation in vector capacity in field populations and about the effects of this variation on bluetongue transmission (77).

## *Vector Competence*

*C. variipennis* transmits bluetongue viruses, African horse sickness virus, akabane virus, and epizootic hemorrhagic disease viruses (7, 19, 22, 42, 52, 60, 61). Laboratory studies have not provided evidence for transovarial transmission of bluetongue viruses from infected *C. variipennis* females to their progeny (47, 86). Therefore it is unlikely that transovarial transmission is a major overwintering mechanism for the virus when adult vectors are not active. Information concerning variation in the ability of infected *C. variipennis* to transmit bluetongue virus is limited. *C. variipennis sonorensis,* containing 2.7–5.1 $\log_{10}$ $TCID_{50}$ (tissue culture infectious doses) per fly regularly transmitted bluetongue virus, while flies with $\leq 2.5$ $\log_{10}$ $TCID_{50}$ did not (41). However, the difficulties in determining transmission rates in the laboratory have prevented evaluations of transmission variation among field populations, using different serotypes and viruses.

SUSCEPTIBILITY TO INFECTION More information is available on *C. variipennis sonorensis* susceptibility to infection with the bluetongue viruses than for other vector-competence traits. However, most of this information is based on studies of a single laboratory colony, known as the 000, sonora strain, or AA colony (49). The transmission studies cited above used this strain. Studies of AA colony flies showed that bluetongue viruses adsorb to host red blood cells and can be observed inside red blood cells up to two days after a *C. variipennis sonorensis* blood meal (108). The *C. variipennis sonorensis* peritrophic mem-

brane did not prevent virus infection of the midgut epithelium, which may occur in the first few hours after ingestion. Bluetongue-virus replication occurs in midgut cells, and the viral particles exit these cells through the basolateral extracellular membrane into extracellular spaces. Virus infection did not result in *C. variipennis sonorensis* cytopathology (108). In tests of three different bluetongue serotypes infecting another colony of *C. variipennis sonorensis* (2), virus first appeared in midgut cells and then in secondary target tissues, e.g. hindgut, fat body, salivary gland, thoracic muscle, and ovarian tissues, excluding follicles and eggs. Salivary gland involvement is particularly important, since this organ delivers the pathogen to a susceptible host during subsequent blood feedings. Within four days after blood feeding, bluetongue virus can be detected in *C. variipennis sonorensis* salivary glands, in salivary gland cytoplasm, in plasma membranes of acinar cells, extracellularly, and within cisternae of vacuoles and endoplasmic reticulum (87). Similar information on bluetongue-virus replication in other subspecies, populations, and other *Culicoides* spp. is lacking.

A key feature of *C. variipennis* infection with bluetongue virus is the likely interference via the mesenteron or gut barrier. *C. variipennis* that were intrathoracically inoculated with bluetongue virus showed higher infection rates than those fed virus via a blood meal (85). Infection rates for intrathoracically inoculated *C. variipennis* have approached 100%, even in colonies that exhibited only 30% infection following ingestion of an infected blood meal (45, 116). In all likelihood, a gut barrier prevents some *C. variipennis* from becoming infected through blood meals. In contrast, inoculation bypasses the midgut, so the infection rates of inoculated insects are higher. The nature of this gut barrier or its role in determining infection of *C. variipennis* field populations is unknown.

In addition to environmental circumstances, *C. variipennis* susceptibility to infection with bluetongue viruses depends on several factors: the subspecies, the population, and the strain of *C. variipennis;* the strain of the virus; any circumstances that may alter the physiologic condition of the insect; the temperature of extrinsic incubation; and the numbers of infectious virions in the blood meal. Virogenesis proceeds much faster, and individual bluetongue-infected *C. variipennis sonorensis* tended to have more virus antigen when incubated at higher temperatures (82). More *C. variipennis sonorensis* females fed with blood meals containing $\geq 10^6$ pfu/ml were infected than those fed on lower concentrations, and no flies were infected from blood meals containing $\leq 10^{-4}$ pfu/ml (46). Susceptibility to infection varies with nutritional status of the larvae: Poor larval nutrition and crowding resulted in small *C. variipennis sonorensis* females that were more susceptible than larger females (WJ Tabachnick, unpublished observations). Different groups from the same generation of the AA colony showed significant variation in BLU-4 infection rates, which

casts doubt on the accuracy of laboratory assessments of vector competence (41). AA colony showed stable infection rates of ~30% with BLU-11 and -17 for several years (48, 49). These observations are consistent with findings that *C. variipennis* infection rates depend on the insect strain, virus isolate, and serotype (50). The infection rates of two *C. variipennis* colonies differed with each of the US serotypes. However, because differences depended on the serotype, the response of either colony to one serotype could not be predicted based on the response to another (67).

Different factors influencing *C. variipennis*–bluetongue virus interactions probably cause infection rates to vary among different insect strains and different viruses. The absence of exotic bluetongue viruses, e.g. Central American–Caribbean bluetongue serotypes, from North America may result from different vector competence and capacity levels of *C. variipennis* for these serotypes—these characteristics need to be evaluated in North American vectors. An understanding of the variety of factors influencing vector competence will require information on the underlying mechanisms, e.g. functional interactions between insect and virus proteins and the effects of environmental factors.

POPULATION HETEROGENEITY Although the specific factors that influence susceptibility to bluetongue infection remain unknown, susceptibility certainly

**Table 1** Susceptibility to infection by bluetongue viruses in *Culicoides variipennis* populations from different US states, 1978–1990[a]

| State | Number of populations | Av. no. insects tested/ population ± SE | Av. % infected insects/population ± SE | Subspecies |
|---|---|---|---|---|
| New York | 5 | 313 ± 2.2 | 2.7 ± 0.4 | *variipennis* |
| New Jersey | 2 | 154 ± 95.0 | 0.6 ± 0.6 | *variipennis* |
| Maryland | 3 | 175 ± 75.0 | 1.0 ± 1.0 | *variipennis* |
| Virginia | 1 | 617 | 0.8 | ?? |
| Montana | 1 | 123 | 4.1 | ?? |
| Missouri | 2 | 530 ± 189.0 | 3.2 ± 0.8 | ?? |
| Nebraska | 4 | 33 ± 9.8 | 24.2 ± 6.9 | *sonorensis* |
| Colorado | 12 | 192 ± 55.9 | 8.9 ± 1.6 | *sonorensis* |
| Oregon | 1 | 29 ± | 27.6 ± | *sonorensis* |
| California | 14 | 157 ± 21.2 | 22.5 ± 2.9 | *sonorensis* |
| California | 2 | 308 ± 143.0 | 1.2 ± 1.1 | *occidentalis* |
| Nevada | 2 | 24 ± 13.0 | 11.3 ± 2.2 | *sonorensis* |
| New Mexico | 1 | 44 | 54.6 | *sonorensis* |
| Utah | 1 | 74 | 14.8 | *sonorensis* |
| Texas | 2 | 70 ± 58.0 | 30.1 ± 11.6 | *sonorensis* |
| Florida | 3 | 34 ± 26.7 | 26.1 ± 13.5 | *sonorensis* |

[a] RH Jones & WJ Tabachnick, unpublished observations.

varies among different subspecies and populations of *C. variipennis* (50). Table 1 shows infection rates with US bluetongue serotypes 2, 10, 11, 13, and 17 for field populations sampled throughout the United States (RH Jones & WJ Tabachnick, unpublished observations). *C. variipennis variipennis* and *C. variipennis occidentalis* are less susceptible than *C. variipennis sonorensis.* Infection of *C. variipennis sonorensis* populations varied from 1.6% in Weld County, Colorado, to 54.6% in Eddy County, New Mexico (RH Jones, unpublished observations). These rates differ for different viruses, and infection with any given serotype does not generally correlate to the rate for other serotypes.

The average infection rate of *C. variipennis* populations from a given state and the seroprevalence of bluetongue antibody in cattle from that state appear strongly correlated (121). This observation is consistent with the hypothesis that the presence of competent *C. variipennis sonorensis* determines bluetongue distribution in the United States. Although vector competence varies greatly within *C. variipennis sonorensis, C. variipennis variipennis* exhibits consistently low susceptibility to infection with US bluetongue-virus serotypes. Of *C. variipennis occidentalis,* only populations from Borax Lake and the Salton Sea in California have been tested for bluetongue-virus susceptibility to infection, and these groups were generally refractory. Other *C. variipennis occidentalis* populations must be tested to determine whether any show higher infection rates. *C. insignis* in southern Florida has infection rates of 20.0–60.5% and can transmit bluetongue virus in the laboratory. This species is likely the predominant bluetongue vector in south Florida (124).

## *Culicoides variipennis and Bluetongue Epidemiology*

Several factors relating bluetongue epidemiology in North America to *C. variipennis* distributions are apparent: (*a*) Bluetongue-virus transmission is virtually absent in the northern United States despite the presence of *C. variipennis variipennis.* (*b*) *C. variipennis sonorensis* is the subspecies in endemic regions of the United States. (*c*) Only BLU-2, -10, -11, -13, and -17 have been observed in the United States, and BLU-2 occurs in isolated instances in the south. (*d*) Bluetongue-virus transmission has been virtually absent from Canada, except in the Okanagan Valley, despite the presence of *C. variipennis* sensu lato (*e*) The epidemiology has been stable despite animal movement and the potential for migration of infected *Culicoides* spp.

*C. variipennis sonorensis* is the primary North American vector of the bluetongue viruses. *C. variipennis variipennis* should not be considered a vector of bluetongue viruses because (*a*) it has a low susceptibility to infection in the laboratory; (*b*) no viruses have been isolated from field-collected insects; (*c*) in regions where it is the only *C. variipennis* subspecies, bluetongue transmission to ruminants has not occurred; and (*d*) environmental conditions

in regions where it occurs reduce vector capacity for long periods—for example, low temperatures increase extrinsic incubation period and prolong the gonotrophic cycle, and lower densities reduce biting rates. The stable 20-year absence of bluetongue in the northeastern United States can only be explained by the nonvector status of *C. variipennis variipennis.* The distributions of *C. variipennis sonorensis* are critical for determining North American regions at risk for bluetongue. In addition, *C. variipennis sonorensis* populations probably sporadically reside in dynamic transition regions, where the fly may extend its range owing to temporary environmental conditions. These populations may cause the low levels and irregular instances of bluetongue transmission seen in such states as Indiana, Ohio, and Virginia (FR Holbrook, personal communication).

The vector status of *C. variipennis occidentalis* is less certain. The Borax Lake population has low susceptibility in the laboratory (Table 1), and bluetongue viruses have not been isolated from this population. However, this subspecies exists sympatrically with *C. variipennis sonorensis* in areas of the western United States where the bluetongue viruses are endemic. Until molecular genetic markers became available, identifying sources of viral isolates from members of the *C. variipennis* complex in western field collections was difficult. *C. variipennis occidentalis* is probably not a major North American vector of the bluetongue viruses, but this supposition must by confirmed by studies using genetic markers to identify field populations and vectors during epizootics.

Canadian bluetongue epizootics likely resulted from incursions of infected *C. variipennis sonorensis* into the Okanagan Valley from the United States or from the importation of viremic ruminants, from which resident *C. variipennis sonorensis* obtained viruses they transmitted to indigenous cattle. Little information is available regarding *C. variipennis* distributions in Canada. *C. variipennis variipennis* occurs in Ontario (118), and probably in the eastern provinces, where *C. variipennis sonorensis* is unlikely. Although *C. variipennis sonorensis* has been collected in the Okanagan Valley of British Columbia and in southern Alberta (FR Holbrook, personal communication), its distribution to the east and north is unknown. The distribution of *C. variipennis sonorensis* in Canada is important because, although some *C. variipennis sonorensis* populations may not be efficient vectors, the current limited information indicates that any region with *C. variipennis sonorensis* is at risk for bluetongue-virus transmission during the insect season.

Our ability to evaluate, predict, and perhaps interrupt the vector potential of *C. variipennis,* as well as to determine regions at risk for bluetongue-virus transmission, depends on the following: (*a*) valid distributions of the subspecies, (*b*) knowledge of genetic control mechanisms responsible for vector capacity and competence, (*c*) ability to analyze populations for genes control-

ling vector capacity and vector competence, and (*d*) information on environmental factors that contribute to variation in vector capacity (119–121).

### *Genetics of Culicoides variipennis Vector Competence*

Investigations on the genetic control of *C. variipennis* susceptibility to infection with bluetongue virus demonstrated the presence of a single controlling gene in two laboratory colonies. Strains of highly susceptible and resistant *C. variipennis sonorensis* were selected from the AA and AK colonies (48, 116). Crosses between susceptible and resistant lines provided evidence for a major locus and a modifier controlling susceptibility (48). Similar studies of colony lines showed that the major controlling locus acted via a maternal effect and paternal imprinting. That is, the mother's genotype determined the progeny phenotype, and the paternal gene was always dominant in offspring (116). This inheritance pattern allowed construction of isogenic pools of flies and identification of a candidate controlling protein that was used to isolate a cDNA clone for sequencing to determine function (KE Murphy & WJ Tabachnick, unpublished observations). Once the candidate gene is identified, further studies will be necessary to determine its role in controlling vector competence variation in field populations. Vector competence is a complex trait, and consequently, it is likely that several genes and various interactions with environmental factors control variation within the species (120).

Genetic mapping studies using DNA molecular markers may in future help us identify other *C. variipennis* vector-competence and vector-capacity genes (99, 120). The long-term goals of these studies are the identification and analysis not only of these genes, but also of the environmental factors that influence them in different *C. variipennis* populations. This information will allow us to (*a*) identify the conditions enabling bluetongue-virus transmission; (*b*) interrupt transmission using releases of genetically altered, resistant *C. variipennis;* and (*c*) reduce vector capacity by changing environmental conditions that affect vector phenotypes.

## CONCLUSIONS

The absence of bluetongue virus from the north and northeastern regions of the United States, temporary incursions into Canada, and the presence of only five serotypes in the United States are consistent with the predominant role of the members of the *C. variipennis* complex in transmission. We are only beginning to appreciate the complexities of arthropod-pathogen interactions. Indeed, the study of *C. variipennis* and bluetongue epidemiology in North America involves many issues common to studies of human and animal arthropod-borne pathogens. Investigations of *C. variipennis* and bluetongue virus show the critical nature of vector-virus interaction that must be understood to

predict vector populations and geographic regions at risk for disease. The results may lead to novel biological control strategies, as opposed to chemical measures, to interrupt pathogen transmission and reduce the effects of disease on animal populations.

Bluetongue in North America largely depends on the distribution of *C. variipennis sonorensis*. Based on bluetongue epidemiology and current vector distributions, it is unlikely that the northern United States and large portions of eastern Canada are at risk for bluetongue-virus transmission.

Regulators establishing policies for animal movement should consider the current situation but must also consider the potential for changes in epidemiology. Caution is warranted. For instance, *C. variipennis variipennis* is probably not a bluetongue-virus vector. However, we do not understand genetic and environmental control mechanisms and the effects of new serotypes or viral variants on vector competence and capacity. Thus, vector status could change. The United States must continue to monitor animal populations in its bluetongue-free areas (130). Once we know the factors responsible for transmission, we can assess the competence of vectors in other regions. For example, we could determine the actual risk for bluetongue-virus transmission by European *Culicoides* spp.

The future for using vector-pathogen information for more effective control of arthropod-borne pathogens is promising. The information reviewed in this chapter can serve as the foundation for efforts to reduce the effect of bluetongue disease on national economies. Regionalization within countries according to the presence of bluetongue virus vectors can reduce unnecessary animal-health regulations and increase opportunities for international trade.

Molecular-biology experiments are under way to determine the mechanisms of action of arthropod traits influencing pathogen transmission. The arthropod-pathogen interactions between *C. variipennis* and bluetongue viruses, *Aedes aegypti* and dengue or yellow fever viruses, or *Anopheles* spp. and the malaria pathogen may even share similarities that will afford opportunities for general control strategies. The first step is to identify the underlying vector-pathogen interactions for different systems, the controlling genes, and the effects of the environment.

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