ARTICLE

Seasonal Transmission of Bluetongue Virus by *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a Southern California Dairy and Evaluation of Vectorial Capacity as a Predictor of Bluetongue Virus Transmission

ALEC C. GERRY,[1] BRADLEY A. MULLENS, N. JAMES MACLACHLAN,[2] AND JAMES O. MECHAM[3]

Department of Entomology, University of California, Riverside, CA 92521

J. Med. Entomol. 38(2): 197–209 (2001)

ABSTRACT Vectorial capacity of *Culicoides sonorensis* Wirth & Jones for the transmission of bluetongue (BLU) virus was examined at a southern California dairy from January 1995 to December 1997. Insects were collected one to two times per week in five CDC-type suction traps (without light) baited with CO_2 at a constant release rate of 1,000 ml/min. BLU virus was detected in midges collected from May through December with an estimated overall infection rate of 0.08%. The BLU virus infection rate of field-captured midges was not correlated with sentinel calf seroconversions to BLU virus. Sentinel calf seroconversions were highly seasonal, occurring from August through November with most calves seroconverting during September and October. Vector competence of field-collected nulliparous flies fed a locally acquired serotype of BLU virus in the laboratory was stable among years (17–23%). Vectorial capacity was strongly correlated with BLU virus transmission (measured by sentinel calf seroconversions) during 1995 and 1996, but not during 1997. Host biting rate estimated for traps nearest to the sentinel calves was the index best correlated with BLU virus transmission for all study years and was most highly correlated with sentinel seroconversions 4 wk later. The utility of vectorial capacity and its component variables is discussed for this system.

KEY WORDS *Culicoides sonorensis*, vectorial capacity, bluetongue virus, infection rate, cattle

THE PRIMARY VECTOR of bluetongue (BLU) virus to ruminants in the United States is the biting midge *Culicoides sonorensis* Wirth & Jones (Foster et al. 1963, Luedke et al. 1967, Holbrook et al. 2000). This midge is found predominantly in the western and southern portions of the United States, and is especially prevalent in association with livestock and polluted waters (Holbrook and Tabachnick 1995).

Seroprevalence data demonstrate a very high level of domestic ruminant exposure to BLU virus in the western and southern United States (Metcalf et al. 1981, Osburn et al. 1981). Seroprevalence rapidly declines in a marked gradient toward the east and north of the United States with essentially no transmission occurring in the north-central and northeastern United States (Metcalf et al. 1981). This cline in exposure is presumed to reflect high vectorial capacity of *C. sonorensis* where BLU virus is transmitted at high levels (Jones et al. 1981, Gibbs and Greiner 1988).

Vectorial capacity is an estimate of the relative capability of a vector population to transmit a disease agent to a population of susceptible hosts (Garrett-Jones 1964, Dye 1986, Reisen 1989) and is defined by the following formula:

$$C = ma^2 V p^n / -\log_e p,$$

where C = vectorial capacity, ma = the host biting rate in bites per host per day, a = host preference/the length of time between bloodmeals, V = vector competence (suitability of the vector population for pathogen infection and transmission), p = the daily probability of survival of the vector, and n = the extrinsic incubation of the disease agent (the number of days that pass between infection of the vector insect and the time when that insect is capable of transmitting the disease agent to a host).

Vectorial capacity is strictly defined as the daily rate at which future inoculations arise from a currently infective case (Garrett-Jones 1964, Dye 1986). However, vectorial capacity and similar measurements on vector populations are likely to have little absolute value due to the compounding of bias and error associated with each of the model components (i.e., actual vectorial capacity cannot be determined) (Dye 1992).

Vectorial capacity, though biased, would be expected to change proportionally with the pathogen

Studies using calves were done in accordance with University of California, Riverside, Animal Use Protocol A-9407013.

[1] Current address: California Department of Health Services, Vector-Borne Disease Section, 5957 S. Mooney Boulevard, Visalia, CA 93277.

[2] Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616.

[3] Arthropod-Borne Animal Diseases Research Laboratory, USDA-ARS, Laramie, WY 82071.

0022-2585/01/0197–0209$02.00/0

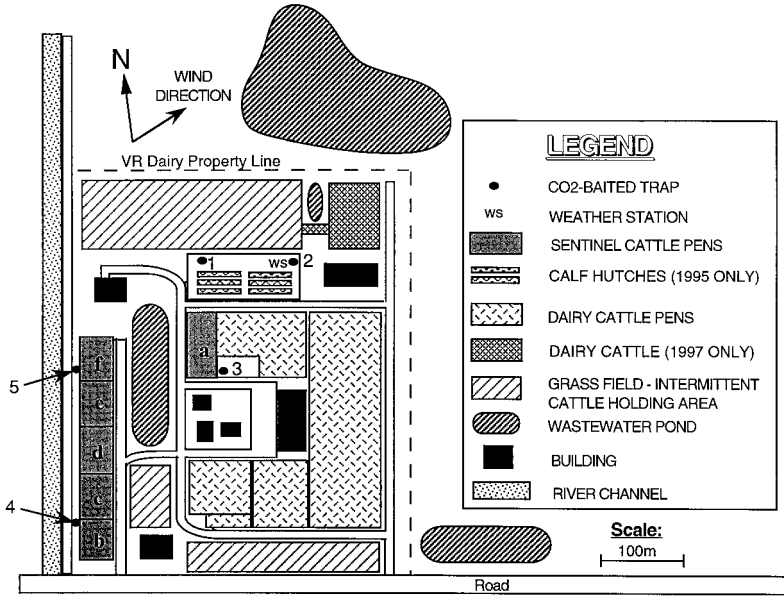

Fig. 1. Layout of the dairy study site. The dairy was centrally located in the Chino Dairy Preserve, San Bernardino County, CA.

transmission rate and therefore would serve as a relative measure of transmission. Used in this way, errors associated with the calculation of vectorial capacity are reduced, as measurements would be biased in the same way. Vectorial capacity may therefore be most useful as a comparative index of transmission over time, from site to site, and between vector species (Dye 1986).

Dye (1986) suggested that a satisfactory comparative index of pathogen transmission need only contain the dominant variables of vectorial capacity. However, the variables that best predict pathogen transmission may differ for each epidemiological situation, and calculation of each of the components of vectorial capacity might be justified in certain situations. In this way, their effects on actual pathogen transmission to vertebrate hosts can be assessed individually and in combination. A vector population lacking somewhat in one component of vectorial capacity may still transmit a disease agent if other components are sufficiently high. This has been demonstrated in Australia where *C. brevitarsis* Kieffer is considered a primary vector of BLU virus due to a very high host biting rate in spite of an exceptionally low vector competence (an infection rate <0.5%) (Standfast et al. 1985).

The Chino Basin Dairy Preserve in southern California is ideal to study the predictive value of the vectorial capacity model with regard to BLU virus transmission. Nearly 100% of the *Culicoides* spp. collected in CO_2-baited traps in this region are *C. sonorensis* (B.A.M., unpublished data) and host-seeking females have been collected throughout the year (Mullens 1985, Gerry and Mullens 2000). Cattle are by far the dominant large animals in this region, and thus most *C. sonorensis* would be expected to feed on this host. Compared with many vector species, parity of *C. sonorensis* is relatively simple to assess via external visual examination (Dyce 1969, Akey and Potter 1979). Additionally, serological surveys of slaughter cattle in southern California have demonstrated a high level of BLU virus exposure in ruminants (Metcalf et al. 1981, Osburn et al. 1981, Stott et al. 1985).

The objective of the current study was to determine each of the components of vectorial capacity for *C. sonorensis* transmission of BLU virus over a 3-yr period at a site expected to have high levels of BLU virus transmission. The component variables of vectorial capacity were then compared with sentinel cattle seroconversions to evaluate their usefulness as predictors of infection prevalence at the study site.

Materials and Methods

Study Area. Studies were conducted at a privately owned dairy located in the Chino Basin dairy preserve of southern California (western Riverside and San Bernardino counties). The dairy preserve is ≈75 km^2 and has >250,000 milking cows. Ponds for wastewater disposal, which provide larval habitat for *C. sonorensis*, are located in close proximity to cattle on nearly all the dairies in this region (Mullens 1989). A covered weather station containing a battery-operated hygrothermograph was placed on site to measure daily fluctuations in temperature and humidity.

Insect Collections. CDC-type miniature suction traps (John W. Hock, Gainesville, FL), with the light removed, were placed at five locations around the dairy to provide coverage of the entire dairy (Fig. 1). Three traps were in close proximity to sentinel calves (traps 3–5). Traps were baited with CO_2 gas released

from a tank with a two-stage regulator at a rate of 1,000 ml/min. This rate is roughly equivalent to the amount of CO_2 produced by a nearly grown Holstein heifer (Roberts 1972). Traps were deployed 2 h before sunset and removed 2 h after sunrise the following day. Host-seeking by female *C. sonorensis* falls mainly within this period (Nelson and Bellamy 1971, Barnard and Jones 1980, Akey and Barnard 1983, Linhares and Anderson 1990, Mullens 1995).

Trapping was conducted continuously from January 1995 through December 1997. Trapping was conducted weekly during winter and spring (January–June) and twice per week during summer and fall (July–December) when midges were more abundant. Summer and fall were presumed to be the seasons of greatest BLU virus transmission (Osburn et al. 1981, Loomis et al. 1985, Stott et al. 1985, Gibbs and Greiner 1994).

***Culicoides* Infection Rate.** Insects captured in the suction traps were returned to the laboratory, anesthetized with CO_2, and placed on a chill table to be sorted and counted. Midges were sorted by sex and females were further sorted by parity status (Dyce 1969, Potter and Akey 1978, Akey and Potter 1979). Transovarial transmission of BLU virus by *C. sonorensis* has not been shown (Jones and Foster 1971a, Nunamaker et al. 1990, Fu et al. 1999), and therefore only parous females would be expected to have acquired BLU virus.

Parous females were placed in microcentrifuge tubes in pools of 10 or fewer, and placed in a Revco freezer at −74°C. Insect pools were then assayed by antigen-capture enzyme-linked immunosorbent assay (ac-ELISA) (Mecham et al. 1990) for the presence of BLU virus. The ac-ELISA used an anti-BLU virus monoclonal antibody (MAb) (1AA4.E4, USDA-ABADRL, Laramie, WY) (1:100) specific for an antigenic determinant of VP7 that is common to BLU virus serotypes BLU-2, 10, 11, and 17, but not to BLU-13 or the closely related strains of epizootic hemorrhagic disease (EHD) virus. This MAb therefore cannot detect midges infected with BLU-13. Assay plates were read using an automated microplate reader (model EL311sx, Bio-Tek Instruments, Winooski, VT).

Negative control insect pools (*C. sonorensis* colony nullipars) and positive control insects (*C. sonorensis* colony nullipars intrathoracically injected with BLU-10) were added to each assay plate. A stock of locally acquired BLU-10 (1.1×10^6 pfu/ml) (10O90Z, de Mattos et al. 1994) was stored at UCR at −74°C for use throughout the study. The primary barriers to BLU virus dissemination in *Culicoides* are bypassed when midges are intrathoracically injected with virus, leading to near 100% infection of injected midges (Mellor 1990, Tabachnick 1996, Fu et al. 1999).

Studies at the Arthropod-Borne Animal Diseases Research Laboratory (ABADRL) showed that ELISA absorbance values are significantly correlated to BLU virus titer determined by plaque assay (Mecham et al. 1990). During the current study, assays were conducted at the University of California at Riverside (UCR). To account for inherent differences between laboratories (due to variation in equipment and chemical stock), ac-ELISA absorbance values at the UCR laboratory were standardized with those at ABADRL.

UCR laboratory-reared (AA strain) *C. sonorensis* were fed (Hunt and McKinnon 1990) or intrathoracically injected with BLU-10, incubated for 14 d at room temperature (28 ± 2°C), and survivors were tested for the presence of BLU virus using the ac-ELISA. Midge homogenate samples ($n = 40$) that represented a wide range of absorbance values (0.106–2.870) were coded and sent on wet ice to ABADRL for comparative (blinded) ac-ELISA testing.

The natural infection rate (IR) of the *C. sonorensis* field population was determined for each 2-wk period by the following formula:

$$IR = (\textit{number positive pools}/\textit{number parous midges tested}) \times \textit{parity rate},$$

which assumes that only one midge was infected per positive pool tested. The entomological inoculation rate (EIR) was calculated as the product of the natural infection rate and the host biting rate.

Sentinel Seroconversion. Each February, a minimum of 88 calves born at the study site were selected to serve as sentinels of BLU virus transmission at the dairy. Sentinel calves were between 6 and 8 mo old and free of BLU virus antibodies (tested as below) at the time of selection. Calves were given numbered ear tags to identify each individually. Sentinel calves were bled monthly through July, and then every 2 wk through the month of December. Blood was collected by jugular venipuncture into a 10 ml vacuum blood collection tube. Blood collection tubes were returned to the laboratory and centrifuged at $514 \times g$ for 30 min. Serum was removed from each tube, placed into 2-ml cryovials, and frozen at −74°C.

Sera were tested for the presence of BLU virus antibodies using the Blueplate Special competitive (c-) ELISA (Diagxotics, Wilton, CT) as per the manufacturer's instructions. Sera from each c-ELISA-positive sentinel calf were sent to the National Veterinary Services Laboratory (Ames, IA) to determine the serotype of infecting virus by serum neutralization. A serum neutralization dilution titer ≥1:40 to a specific BLU virus serotype was considered indicative of exposure to that serotype (Thomas 1985, Rossitto and MacLachlan 1992, Singer et al. 1998).

Vector Competence. Vector competence is a measure of the ability of an insect vector to acquire, allow replication of, and transmit virus. For this study, the infection rate of field-collected nulliparous midges fed an infective bloodmeal in the laboratory was measured as a relative index of vector competence.

Field-collected nulliparous midges were fed (Hunt and McKinnon 1990) on commercially obtained, pathogen-free sheep blood (Advanced Scientifics, Millersburg, PA) to which BLU-10 was added to a final concentration of 1.1×10^5 pfu/ml. Female *C. sonorensis* ingest ≈0.1 μl of blood during a bloodmeal (Foster and Jones 1979). After feeding (30 min to 1 h), midges were anesthetized with CO_2, placed on a chill

table, and fully engorged midges were separated and placed into a 237 ml holding container supplied with a vial of 10% sucrose. Holding containers were placed inside a plastic secondary containment box containing a 100-ml beaker with NaCl-saturated water to maintain ≈75% humidity (Winston and Bates 1960). Insects were incubated at 28 ± 2°C for 6–11 d after which survivors were placed individually in 1.5-ml microcentrifuge tubes and frozen at −74°C. Individual insects were tested for infection with BLU virus by ac-ELISA.

Host Preference. Recently fed *C. sonorensis* midges were sampled by truck-mounted net trap (Nelson and Bellamy 1971). The trap was made of fine mesh net with a 1.3-m-diameter opening that tapered into a rigid detachable insect collection bag and was mounted on the roof of a truck, ≈2 m off the ground. Insects were collected while repeatedly driving a 6.3-km loop course at 35–40 km/h near dusk in the vicinity of the study dairy during August of 1995 and 1996. Insects captured in the truck-mounted trap were returned to the laboratory and placed in a freezer (-20°C) overnight. The next day, blood-engorged midges were separated, placed individually in microcentrifuge tubes, and frozen at −74°C.

Bloodmeal sources of individual *C. sonorensis* were identified by agar-gel precipitin test (Crans 1969). Midge homogenates were tested against the following hosts: cow, horse, dog, rabbit, and a saline control.

Vectorial Capacity. Mean values for each 2-wk period were determined for each of the components of vectorial capacity to compare these components to sentinel seroconversion data. Mean monthly values and details of seasonal and spatial variability for these components have been previously reported for this study site (Gerry and Mullens 2000), and raw data are available (Gerry 1999).

Comparisons were limited to data from June through November when field temperature was >18°C due to the lack of representative data for BLU virus virogenesis at lower temperatures (see Gerry and Mullens 2000). The June through November period encompassed the BLU virus transmission season, and midge abundance was high enough to ensure robust parity rates for calculation of daily survivorship.

Host biting rate (ma) was determined by the mean number of midges captured per trap night adjusted by the relationship of CO_2-baited trap collections to actual biting rate on calves at this site. Abundance of *C. sonorensis* measured by CO_2-baited trap capture at this dairy site has been shown to be significantly correlated ($r = 0.82$) with collections from calf-baited traps (Mullens and Gerry 1998). A correction factor of 3.7 times the CO_2-baited trap catch approximates the number of bites a calf would experience under similar conditions.

The length of the gonotrophic cycle is important both as a measure of the frequency of blood-feeding and as a component of the calculation of daily survivorship. At our study site, the majority of the gonotrophic cycle was likely composed of oogenesis due to the abundance and proximity of cattle hosts and dairy wastewater ponds (i.e., host availability and oviposition sites were not limiting). The length of the gonotrophic cycle was therefore estimated by the time to oviposition following a bloodmeal. A regression equation ($y = -1.98 + 0.07217x + 2516.65x^{-2}$) has been determined such that the time to oviposition (y) can be calculated based on temperature (x) (Mullens and Holbrook 1991).

Mean daily survivorship for each 2-wk period was estimated from mean parity rates of captured females using the method of Davidson (1954). Thus, p is estimated by $P^{1/u}$, where p is the daily probability of survival, P is the parity rate of field-captured midges, and u is the length of the gonotrophic cycle. Dye (1990) suggested that using the parous rate to determine survivorship works reasonably well when the aim is to measure comparative change in vectorial capacity.

Data previously reported for the extrinsic incubation period of BLU virus at various temperatures (Foster et al. 1963; Luedke et al. 1967; Foster and Jones 1973, 1979; Chandler et al. 1985; Mullens et al. 1995; unpublished data) and summarized by Gerry and Mullens (2000) were used to calculate a regression equation ($y = -1.03x + 36.79$) such that for a given temperature above 18°C (x), the length of the extrinsic incubation period (y) could be estimated.

Statistics. ELISA absorbance values of BLU virus-positive insect pools collected from July through November (1995 and 1996) were subjected to analysis of variance (ANOVA) using the GLM procedure in Minitab version 11.2 for Windows (Minitab 1996) with year and month as the main effects. Means for significant main effects were separated using Tukey's honestly significant difference test.

The infection rate of field-collected nullipars fed an infectious bloodmeal in the laboratory was analyzed by chi-square analysis to detect differences in the infection rate versus year of capture. Differences in the infection rate by month of midge capture were also analyzed by chi-square analysis for midges captured from July through October of 1996, when a reasonable number of field-collected nullipars engorged on an infectious bloodmeal in the laboratory and survived an appropriate incubation period.

The sentinel calf seroconversion rate was subjected to chi-square analysis to detect differences in the seroconversion rate versus study year. The Julian dates of sentinel calf seroconversions were subjected to analysis by ANOVA using the GLM procedure to detect temporal differences in BLU virus transmission between years.

Vectorial capacity and each of its component variables was correlated with the number of sentinel seroconversions for corresponding 2-wk periods using Pearson correlation. The midge infection rate and the entomological inoculation rate were correlated with sentinel seroconversions due to BLU virus serotypes other than BLU-13 (which was undetectable in the insect assays) for corresponding 2-wk periods. Sentinel seroconversions were then lagged by consecutive

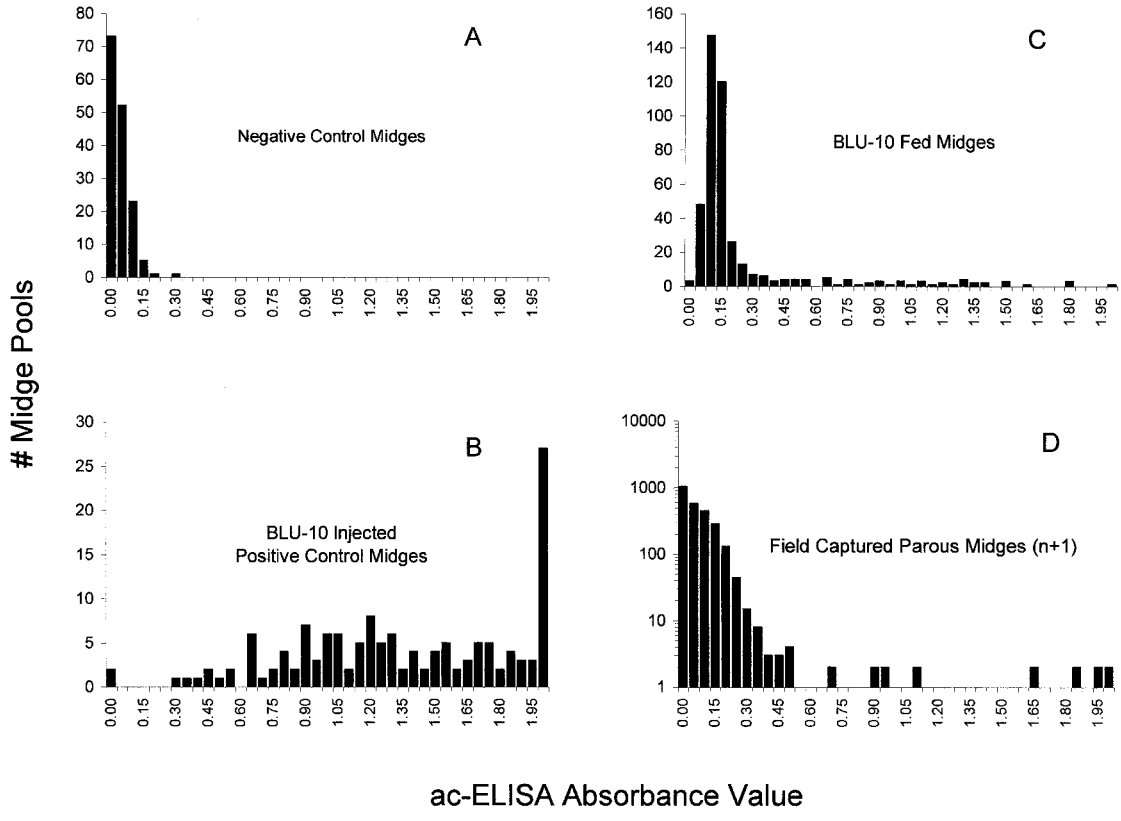

Fig. 2. (A) Number of laboratory-reared (negative control) *Culicoides sonorensis* per ac-ELISA absorbance value range. (B) Number of laboratory-reared *C. sonorensis* injected with BLU-10 (positive control) per absorbance value range. (C) Number of field-captured nulliparous *C. sonorensis* fed a BLU-10 infectious bloodmeal per absorbance value range. (D) Number of field-captured parous *C. sonorensis* per absorbance value range. Column labels indicate the minimum absorbance value for each column range.

2-wk intervals (up to 10 wk) and analyzed at each lag for correlation with these same variables.

Results

***Culicoides* Infection Rate.** There was a strong correlation between the ac-ELISA performed at UCR and at ABADRL ($R^2 = 0.93$, df = 38, $P < 0.01$). At the UCR laboratory, a midge pool was considered BLU virus-positive with an ac-ELISA absorbance value ≥0.250.

The ac-ELISA was a very accurate assay. Mean per plate absorbance values of negative control pools never approached the positive cutoff value, and only one ($n = 155$) negative control pool exceeded the 0.250 cutoff (Fig. 2A). Positive control midges injected with BLU-10 and incubated for 7–14 d at 28 ± 2°C were all clearly positive (absorbance value ≫ 0.250), except for two midges that apparently did not receive virus during the injection process (Fig. 2B). In contrast, absorbance values of BLU virus-fed and field-captured parous midges ranged broadly, but relatively few field-captured parous midge pools had high absorbance values (Fig. 2 C and D).

Overall, 19,645 field-collected parous midges in 3,064 pools were tested for BLU virus (Fig. 3), of which 79 pools tested positive (2.6% of pools or 0.4% of midges tested). BLU virus was detected in midge pools collected from May through December (Fig. 4A). Only one BLU virus-positive midge pool was detected in 1997, therefore the analyses below are restricted to data from 1995 and 1996.

Absorbance values were significantly higher for BLU virus-positive midge pools captured in September (mean = 0.71) than for positive pools captured in July or August (mean = 0.36 and 0.29, respectively), but were not different from positive pools captured in October or November (mean = 0.36 and 0.42, respectively) ($F = 3.18$; df = 4, 64; $P < 0.05$). There was no significant difference in the absorbance values of BLU virus-positive midge pools captured in 1995 versus 1996 ($F = 0.23$; df = 1, 64; $P > 0.05$).

The infection rate of field-collected midges (for 2-wk periods) varied from 0.0 to 2.2%, with substantial variation between study years (Fig. 4B). Although the midge infection rate was not correlated with sentinel seroconversions at any lag (best correlation: $r = -0.034$, df = 76, $P > 0.05$), the entomological inoculation rate was weakly correlated with sentinel seroconversions 4 wk later ($r = 0.267$, df = 76, $P < 0.05$). The mean estimated entomological inoculation rate at

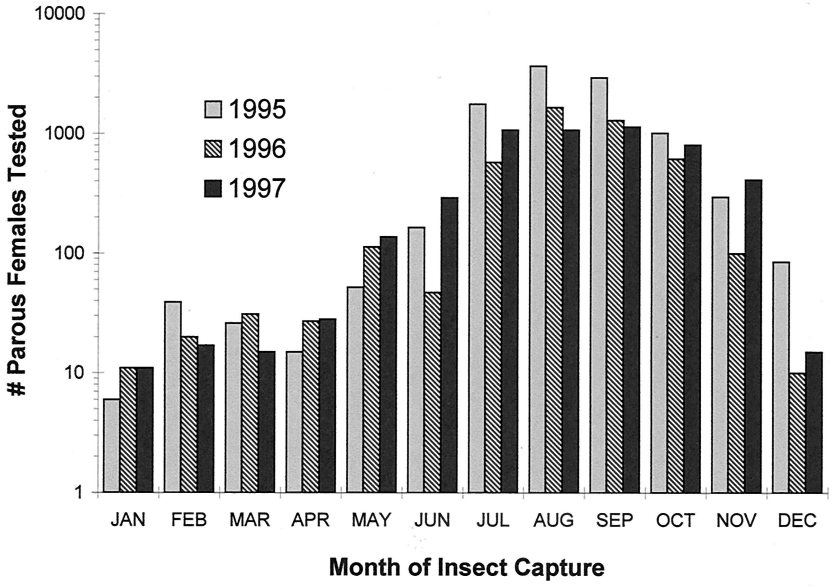

Fig. 3. Total field-captured parous *Culicoides sonorensis* tested for BLU virus presence by ac-ELISA, by year and month of midge capture.

traps nearest the sentinel calves for 1995–1996 was 0.05 infective bites per calf per night, or 19 infective bites per calf per year.

Sentinel Seroconversion. Seroconversions were highly seasonal and restricted to late summer through fall, with one exception. A single calf seroconverted at some time between the end of the 1997 sampling season (5 December) and a postseason sampling on 5 February 1998.

There was a significant difference in the number of sentinel calves seroconverting during each year of the study ($\chi^2 = 11.82$, df = 2, $P < 0.003$) with nearly twice as many seroconversions in 1997 (35 of 93) as compared with 1995 (15 of 85) or 1996 (17 of 88) (Fig. 5). Although BLU virus transmission occurred earlier in 1997 (mean date of sentinel seroconversion was 30 September) compared with 1995 or 1996 (mean date of sentinel seroconversion was 18 October and 16 October, respectively), this difference was not statistically significant ($F = 2.34$; df = 2, 46; $P > 0.05$).

Vector Competence. In general, only a small percentage of field-collected nullipars fully engorged on an infectious bloodmeal in the laboratory. Of those that did fully engorge, half or less survived a reasonable incubation period (6–11 d). Overall, 430 midges engorged and survived an incubation period, of which 85 (20%) became infected (had an ac-ELISA absorbance value ≥0.250).

The infection rate of BLU virus-fed midges versus year of capture was not significantly different ($\chi^2 = 2.183$, df = 2, $P = 0.336$). Infection rates per year were 17, 23, and 17% (1995, 1996, and 1997, respectively). The infection rate versus month of midge capture was also not significant ($\chi^2 = 4.790$, df = 3, $P = 0.188$) for months tested.

Host Preference. Of 109 engorged female *C. sonorensis* collected by truck trap, 106 (97%) had fed on cattle, one on a horse, and two midges failed to produce precipitin bands to any of the anti-host antibodies.

Vectorial Capacity. Vectorial capacity was highest from July through October for each year, but was not correlated with the number of sentinel calves seroconverting at any lag (best correlation: $r = 0.316$, df = 31, $P > 0.05$) (Fig. 6A). Removing 1997 from the analysis, there was a strong positive correlation between vectorial capacity and seroconversions 4 wk later ($r = 0.560$, df = 20, $P < 0.01$).

The host biting rate (all traps) was distinctly seasonal with a single peak occurring each summer and was most positively correlated with sentinel seroconversions 8 wk later ($r = 0.530$, df = 31, $P < 0.05$) (Fig. 6B). However, restricting the analysis to data obtained from traps nearest to the sentinel calves resulted in a higher correlation of host biting rate with sentinel seroconversions, while reducing the best correlation interval to 4 wk ($r = 0.656$, df = 31, $P < 0.01$) (Fig. 6C).

Sentinel seroconversions were generally associated with a host biting rate 4 wk earlier at trap sites nearest the sentinel calves of approximately >70–110 bites/host/d (Fig. 7).

Discussion

***Culicoides* Infection Rate.** The current study examined BLU virus presence in field vectors in detail and over multiple years. Most studies on BLU virus in vectors report a few isolations from large numbers of pooled midges and therefore lack seasonal detail. The

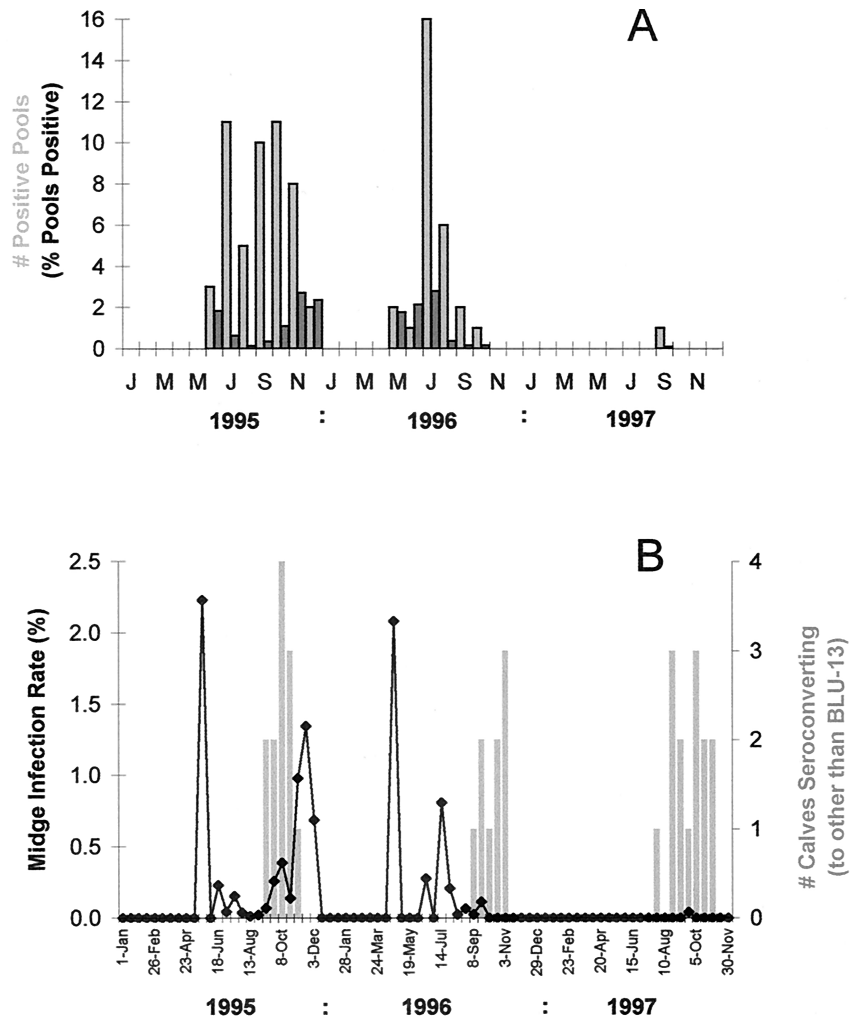

Fig. 4. (A) Number of field-captured parous *Culicoides sonorensis* pools testing positive by ac-ELISA for BLU virus infection and percentage of pools tested that were positive, by month of midge capture. (B) Estimated BLU virus infection rate of *C. sonorensis* compared with sentinel calf seroconversions to BLU virus serotypes other than BLU-13 for each 2-wk period of the study. Sentinel calf seroconversions to BLU-13 were excluded because the ac-ELISA used to determine BLU virus presence in insects could not detect midge infection with BLU-13.

ease of use and low cost of the ac-ELISA are advantageous in conducting large-scale surveys.

The mean BLU virus infection rate of field-captured *C. sonorensis* from 1995 to 1997 was 0.4% of parous flies. With an overall average 20.3% parous rate, the overall field infection rate was 0.08%. This was higher than the infection rate reported at an active BLU virus transmission site in Louisiana, where BLU virus was detected by polymerase chain reaction analysis in only one of 6,072 (0.02%) midges in 381 pools (Wieser-Schimpf et al. 1993). The infection rate at the Chino dairy site was similar to the infection rate indicated by dot-blot procedure (0.06%), by ELISA (0.1%), and by immuno-electron microscopy (0.4%) in 1,800 individual midges collected at an active BLU virus transmission site in northeastern Colorado (Nunamaker et al. 1997).

BLU virus was detected in midges collected from May through December. The first detection of BLU virus in midges collected during 1995 and 1996 was 2–3 mo before the summer midge abundance peak and 3–4 mo before the first sentinel seroconversion. Nevill et al. (1991) reported that BLU virus was regularly recovered from *Culicoides imicola* Kieffer in South Africa before it was detectable in sentinel sheep. Surveillance of vector populations may therefore indicate virus activity months before seroconversion of sentinel animals.

The lack of correlation of sentinel seroconversions with the midge natural infection rate may reflect two problems. First, the association between ac-ELISA absorbance values and the ability of a midge to transmit BLU virus is unknown. Midges with ac-ELISA detectable BLU virus, but with low absorbance values,

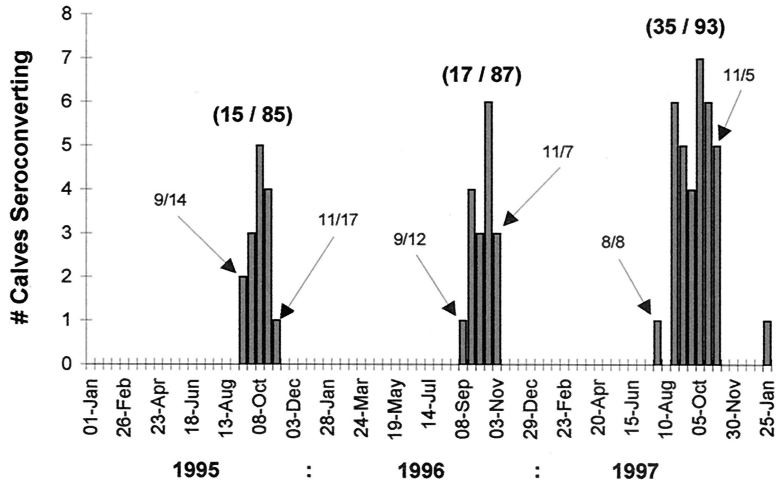

Fig. 5. Number of sentinel calf seroconversions to all serotypes of BLU virus (including BLU-13) detectable by c-ELISA during each 2-wk period of the study.

may not be capable of BLU virus transmission. A relationship between the BLU virus titer of an individual midge and its ability to shed virus in its saliva has been shown (Fu et al. 1999). Second, even with the relatively large numbers of insects collected and processed, the low infection rate resulted in fairly broad and overlapping confidence intervals for each of the 2-wk periods sampled.

BLU Virus Detection Assays. The use of MAb in the ac-ELISA had the substantial advantage of not cross-reacting with the closely related EHD virus (Mecham et al. 1990). The cost, however, was lack of detection of BLU-13, a serotype whose prevalence was thought to be relatively low in the United States (Barber 1979) and in California (Osburn et al. 1981, Loomis et al. 1985, Stott et al. 1985). Details of seroprevalence will be published in the future (unpublished data). However, BLU-13 became the dominant serotype during 1997. A polyclonal antibody, capable of detecting BLU-13, was used on many of the insect pools from 1997 and detected virus in a number of the insect pools that were BLU virus-negative when tested with MAb (Gerry 1999). It is likely these midges were infected with BLU-13. Even so, it is odd that more insect pools from 1997 were not identified as BLU virus-positive using the MAb, as sentinel seroconversions to BLU-11 and 17 were similar to numbers observed during 1995 and 1996.

Relatively few BLU virus-fed midges had absorbance values in the lowest category (0.0–0.049), suggesting that the midges may have become infected with BLU virus, but viral dissemination did not occur. Jennings and Mellor (1987) and Fu et al. (1999) have reported low-level BLU virus infections (perhaps associated with a mesenteron escape barrier or virus dissemination barrier) in a portion of BLU virus-fed midges, whereas high-level disseminated infections occurred in others. The incubation period of BLU virus-fed midges was influenced by the daily survivorship of each cohort. Those cohorts with poor daily survival were frozen for later assay following a shorter period of incubation, although always ≥6 d at 28°C. At this temperature, 6 d is quite adequate for virus to replicate to a high level (Mullens et al. 1995). Still, variation in the incubation period of these midges may have been a factor in the broad spread of their absorbance values above 0.250.

In contrast to the BLU virus-fed midges, a relatively small proportion of the field-collected parous midges that were BLU virus-positive had high absorbance values. High midge mortality in the field would prevent most midges from surviving long enough to allow BLU virus to replicate to high titer. Mean monthly absorbance values for BLU virus-positive midge pools were significantly lowest in July and August, during months when mortality was greatest (Gerry and Mullens 2000). The mean absorbance values were significantly highest during September when most BLU virus transmission was occurring, indicating that a relationship may exist between high viral titer in midges (as measured by the ac-ELISA) and the ability to transmit BLU virus.

Overwintering. Winter temperatures at the dairy site met those necessary for adult midge survival through the winter (Sellers and Mellor 1993, Gerry and Mullens 2000) and midges were active year-round. At low temperatures, BLU virus does not replicate, but may persist at very low levels until higher temperatures are experienced, at which point virogenesis will occur (Mullens et al. 1995). Similar results have been shown for the closely related African horse sickness virus (Mellor et al. 1998). Intensive collecting at this site during winter ought to indicate whether viral overwintering in active adult midges occurs in southern California.

Sentinel Seroconversion. Sentinel seroconversions were distinctly seasonal and occurred from August through mid-November at the dairy. Previous studies in California have shown that isolation of BLU virus from ruminants is also most common from August

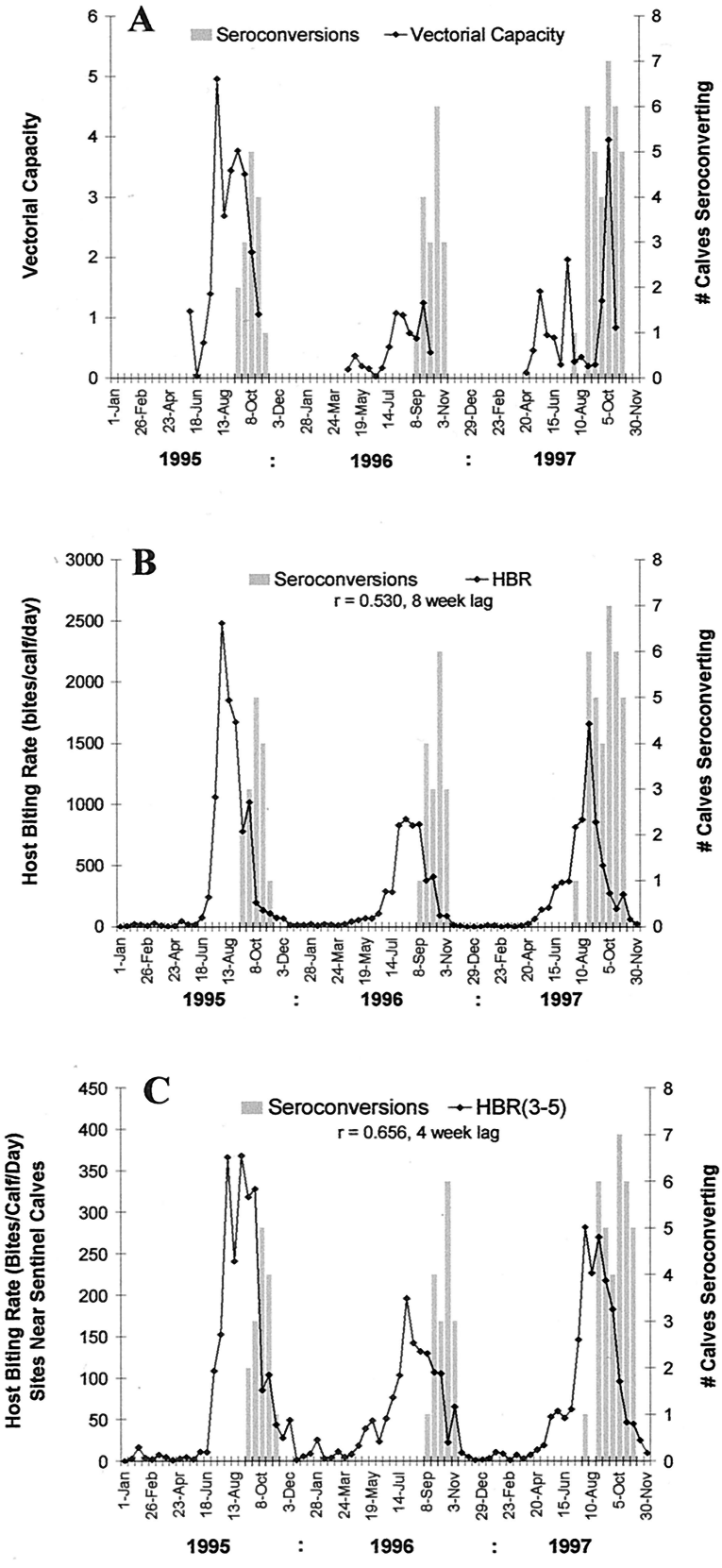

Fig. 6. (A) Vectorial capacity of *Culicoides sonorensis* compared with sentinel calf seroconversions during each 2-wk period of the study. Vectorial capacity for all three study years was not correlated with sentinel seroconversions at any lag. (B) Host biting rate of *C. sonorensis* (from all trap sites) compared with sentinel calf seroconversions during each 2-wk period of the study. Host biting rate was best correlated with sentinel seroconversions 8 wk later (r = 0.530). (C) Hosting biting rate of *C. sonorensis* at trap sites nearest to the sentinel calves (sites 3–5) compared with sentinel calf seroconversions during each 2-wk period of the study. Host biting rate was best correlated with sentinel seroconversions 4 wk later (r = 0.656).

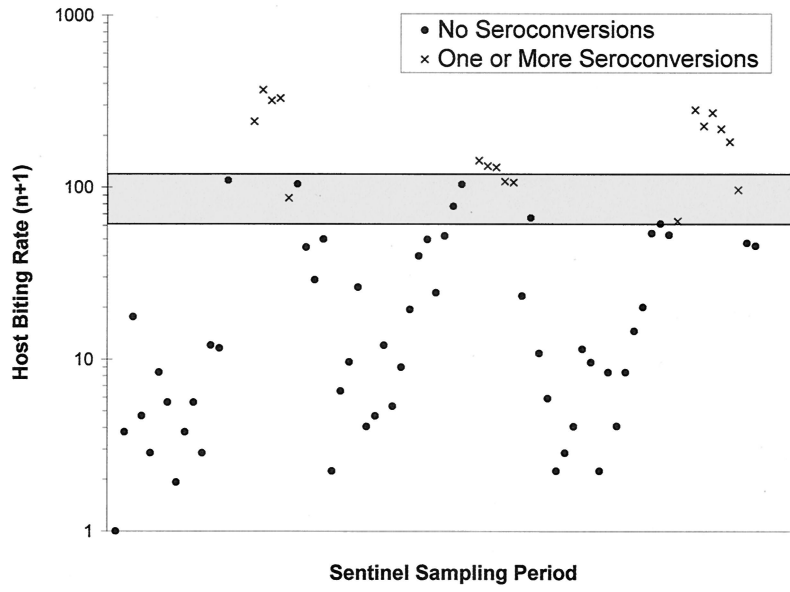

Fig. 7. Relationship of BLU virus transmission with estimated host biting rate for trap sites nearest to the sentinel calves (sites 3–5). Host biting rate was estimated for each 2-wk period of the study and data markers indicate whether one or more sentinel calves seroconverted (×) or no sentinel calves seroconverted (●) to BLU virus 4 wk later. The darkened band indicates a range of host biting rate values above which sentinel seroconversions occurred and below which sentinel seroconversions did not occur 4 wk later.

through November, but virus has been isolated as early as June and as late as December (Osburn et al. 1981).

Mean field temperature was positively correlated with sentinel seroconversions 8 wk later ($r = 0.630$, $df = 31$, $P < 0.01$). In Australia, seroconversion of sentinel cattle was correlated with field temperatures 1 mo earlier (Ward 1996). However, seroconversion of sentinel cattle in Alabama was not correlated with mean daily temperatures (Wright et al. 1993).

Bluetongue infection in cattle has been associated with rainfall 1–3 mo earlier in Australia (Ward and Thurmond 1995, Ward 1996) and total rain days 2 wk earlier in the eastern United States (Wright et al. 1993). In southern California, summer rains generally do not occur, and precipitation from June through mid-fall was negligible for each year of this study. During this study, winter precipitation varied substantially (Gerry and Mullens 2000); however, there appeared to be no relationship between winter precipitation and the number or timing of later sentinel seroconversions. By midsummer, most temporary pools had dried and dairy wastewater ponds provided the only significant larval habitat.

Vector Competence. The 20% infection rate of *C. sonorensis* fed BLU-10 in the laboratory during this study was similar to the 10–30% infection rate experimentally obtained for *C. sonorensis* by Jones and Foster (1971b, 1974, 1978a, 1978b). It is also close to the 22.5% infection rate reported by Tabachnick (1996) for 14 California field populations assayed between 1978 and 1990. The ability of *C. sonorensis* to support viral replication has been shown to be at least partially under genetic control (Tabachnick 1991), which may make this trait relatively stable over time.

Environmental temperature may affect the infection rate of *Culicoides* as it does in some mosquitoes (Kay et al. 1989, Turell 1993). Mellor et al. (1998) demonstrated that raising the rearing temperature of immature *C. nubeculosus* (Meigen) (normally a poor vector) resulted in an increase in infection rate with African horse sickness virus. In this study we saw no evidence that the infection rate of field-collected midges fed BLU virus in the laboratory varied from midsummer through mid-fall.

Vectorial Capacity. Vectorial capacity was used in this study as a relative measure of BLU virus transmission, rather than a quantitative measure of future new infections. It is understood that model components are likely to be biased, and each component will have an error associated with it. However, by using vectorial capacity as a comparative index of BLU virus transmission, error would be minimized because measurements being compared would be similarly biased (Dye 1992).

Vectorial capacity was predictive of future sentinel seroconversions for 1995 and 1996, but not for 1997. The lack of correlation between vectorial capacity and sentinel seroconversions for 1997 was due to a reduction in vectorial capacity that occurred at the start of the BLU virus transmission season. This reduction in vector capacity was principally due to a very low estimate of daily survivorship for this period (Gerry and Mullens 2000).

Vectorial capacity is very sensitive to changes in survivorship, and a poor estimate of daily survivorship will result in a very poor calculation of vectorial capacity. For this study, midge daily survivorship was estimated by the mean parity rate during each 2-wk

period to match the bleeding interval of the sentinel calves. This short period makes our survivorship estimate subject to error because of midge cohort fluctuations. However, estimating survivorship using an eight point running mean (4-wk period) for parity did not improve the correlation of vectorial capacity with sentinel seroconversions (best correlation: $r = 0.161$, $df = 31$, $P > 0.05$), and using a constant mean summer daily survivorship ($P = 0.60$) also failed to produce a significant correlation (best correlation: $r = 0.343$, $df = 31$, $P > 0.05$). In this system, parity was simple to assess, but a more direct survivorship estimate, as derived from mark-release-recapture, might improve the model.

Vectorial capacity was not determined for winter and spring due to the lack of BLU virus extrinsic incubation period data at low temperatures. Vectorial capacity during these seasons is likely low due to a very low host biting rate and the lack of BLU virus virogenesis below 15°C (Mullens et al. 1995). However, if virogenesis does occur, perhaps during periods when temperatures briefly exceed 15°C, vectorial capacity during these seasons may be quite high due to increased midge daily survivorship ($P > 0.90$) (Gerry and Mullens 2000).

The dominant components of vectorial capacity that are the most important in determining transmission intensity vary for different epidemiological situations. Rosenberg et al. (1990) found mosquito survivorship to be the dominant factor in malaria transmission in Thailand. Reisen et al. (1995) suggested that St. Louis and western encephalitis virus transmission was most influenced by shortening the viral extrinsic incubation period without markedly decreasing mosquito survivorship. Host biting rate was the most important factor in determining vectorial capacity of *Anopheles gambiae* Giles for malaria (Bockarie et al. 1995).

The best index of BLU virus transmission at our study site was the host biting rate, which was significantly correlated with sentinel seroconversions 8 wk later at all trap sites and 4 wk later at trap sites nearest to the sentinel calves. A vector generation in summer at this field site is approximately 4 wk (Gerry and Mullens 2000). A 4-wk lag between host biting rate and sentinel seroconversions would be consistent with the estimated 9–10 d extrinsic incubation period of BLU virus during the summer and the 10–20 d required for calf antibody production following infection with BLU virus. The precipitous drop in sentinel seroconversions in November is likely related to the equally precipitous drop in host biting rate that occurred each year in mid-October. Although the gonotrophic cycle length was significantly negatively correlated with sentinel seroconversions 8 wk later ($r = -0.625$, $df = 31$, $P < 0.01$), midge daily survivorship and the probability of surviving the extrinsic incubation period were also significantly negatively correlated with sentinel seroconversions 8 wk later ($r = -0.718$ and -0.402, $df = 31$, $P < 0.01$ and 0.05, respectively). Therefore, these correlations likely reflect the effect of temperature on the host biting rate (Gerry and Mullens 2000). The enormous increase in midge abundance with increased temperatures during summer and early fall appears to outweigh other factors in the transmission of BLU virus.

Host biting rate at trap sites nearest to the sentinel calves was a better predictor of sentinel seroconversions than host biting rate at all trap sites. Midge abundance at these trap sites would be expected to represent most closely the biting intensity experienced by our sentinel calves. Host biting rate is a difficult parameter to measure directly; however, vector abundance in CO_2-baited traps is a relative estimate of host biting rate that is easily measured. In a herd setting, traps at the edge of the animal group probably overestimate the actual average biting rate for individual animals within the group due to encounter dilution and selfish herd effects (see Hart 1997).

During summer, very high host biting rates (up to 2,500 bites/host/d) compensate for low midge survivorship making interruption of BLU virus transmission through vector control difficult. If BLU virus transmission occurs year-round at the dairy and is not annually reintroduced, vector control operations conducted during winter may have more success than those conducted during the summer and fall. Midge abundance is low during the winter, and a reduction in midge survivorship during this time should have a substantial effect on vectorial capacity and virus transmission.

Acknowledgments

We thank M. S. Mulla and W. E. Walton (both of the University of California at Riverside) for reviewing an earlier version of the manuscript. We also thank an anonymous reviewer for a thorough and insightful review. We are grateful to the members of the UCR veterinary entomology laboratory (R. K. Velten, C. E. Szijj, K. A. Luhring, and T. Chin) for their assistance in this study. We appreciate the cooperation of the Van Ryn Dairy where these studies were conducted. The study was funded by USDA-NRICGP Grant No. 94-37204–1092 to B.A.M.

References Cited

Akey, D. H., and D. R. Barnard. 1983. Parity in airborne populations of the biting gnat *Culicoides variipennis* (Diptera: Ceratopogonidae) in northeastern Colorado. Environ. Entomol. 12: 91–95.

Akey, D. H., and H. W. Potter. 1979. Pigmentation associated with oogenesis in the biting fly *Culicoides variipennis* (Diptera: Ceratopogonidae): Determination of parity. J. Med. Entomol. 16: 67–70.

Barber, T. L. 1979. Temporal appearance, geographic distribution, and species of origin of bluetongue virus serotypes in the United States. Am. J. Vet. Res. 40: 1654–1656.

Barnard, D. R., and R. H. Jones. 1980. Diel and seasonal patterns of flight activity of Ceratopogonidae in northeastern Colorado. Environ. Entomol. 9: 446–451.

Bockarie, M. J., M. W. Service, G. Barnish, and Y. T. Touré. 1995. Vectorial capacity and entomological inoculation rates of *Anopheles gambiae* in a high rainfall forested area

of southern Sierra Leone. Trop. Med. Parasitol. 46: 164–171.

Chandler, L. J., M. E. Ballinger, R. H. Jones, and B. J. Beaty. 1985. The virogenesis of bluetongue virus in *Culicoides variipennis.* Prog. Clin. Biol. Res. 178: 245–253.

Crans, W. J. 1969. An agar gel diffusion method for the identification of mosquito bloodmeals. Mosq. News 29: 563–566.

Davidson, G. 1954. Estimation of the survival-rate of Anopheline mosquitoes in nature. Nature (Lond.) 174: 792–793.

de Mattos, C. C., C. A. de Mattos, B. I. Osburn, and N. J. MacLachlan. 1994. Evolution of the L2 gene of field isolates of bluetongue virus serotype 10 from California. Virology 201: 173–177.

Dyce, A. L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. J. Aust. Entomol. Soc. 8: 11–15.

Dye, C. 1986. Vectorial capacity: must we measure all its components? Parasitol. Today 2: 203–209.

Dye, C. 1990. Epidemiological significance of vector-parasite interactions. Parasitology 101: 409–415.

Dye, C. 1992. The analysis of parasite transmission by bloodsucking insects. Annu. Rev. Entomol. 37: 1–19.

Foster, N. M., and R. H. Jones. 1973. Bluetongue virus transmission with *Culicoides variipennis* via embryonating chicken eggs. J. Med. Entomol. 10: 529–532.

Foster, N. M., and R. H. Jones. 1979. Multiplication rate of bluetongue virus in the vector *Culicoides variipennis* (Diptera: Ceratopogonidae) infected orally. J. Med. Entomol. 15: 302–303.

Foster, N. M., R. H. Jones, and B. R. McCrory. 1963. Preliminary investigation on insect transmission of bluetongue virus in sheep. Am. J. Vet. Res. 24: 1195–1200.

Fu, H., C. J. Leake, P. P. Mertens, and P. S. Mellor. 1999. The barriers to bluetongue virus infection, dissemination and transmission in the vector *Culicoides variipennis* (Diptera: Ceratopogonidae). Arch. Virol. 144: 747–761.

Garrett-Jones, C. 1964. The human blood index of malaria vectors in relation to epidemiological assessment. Bull. W.H.O. 30: 241–261.

Gerry, A. C. 1999. Vector capacity of *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) and transmission of bluetongue virus on a southern California dairy. Ph.D. dissertation, University of California, Riverside.

Gerry, A. C., and B. A. Mullens. 2000. Seasonal abundance and survivorship of *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) at a southern California dairy, with reference to potential bluetongue virus transmission and persistence. J. Med. Entomol. 37: 675–688.

Gibbs, E.P.J., and E. C. Greiner. 1988. Bluetongue and epizootic hemorrhagic disease, pp. 39–70. *In* T. P. Monath [ed.], The arboviruses: epidemiology and ecology. CRC, Boca Raton, FL.

Gibbs, E.P.J. and E. C. Greiner. 1994. The epidemiology of bluetongue. Comp. Immunol. Microbiol. Infect. Dis. 17: 207–220.

Hart, B. L. 1997. Behavioral defence. *In* D. H. Clayton and J. Moore [eds.[rsq], Host-parasite evolution: general principals and avian models. Oxford University Press, New York.

Holbrook, F. R., and W. J. Tabachnick. 1995. *Culicoides variipennis* (Diptera: Ceratopogonidae) complex in California. J. Med. Entomol. 32: 413–419.

Holbrook, F. R., W. J. Tabachnick, E. T. Schmidtmann, C. N. McKinnon, R. J. Bobian, and W. L. Grogan. 2000. Sympatry in the *Culicoides variipennis* complex (Diptera: Ceratopogonidae): a taxonomic reassessment. J. Med. Entomol. 37: 65–76.

Hunt, G. J., and C. N. McKinnon. 1990. Evaluation of membranes for feeding *Culicoides variipennis* (Diptera: Ceratopogonidae) with an improved artificial blood-feeding apparatus. J. Med. Entomol. 27: 934–937.

Jennings, D. M., and P. S. Mellor. 1987. Variation in the responses of *Culicoides variipennis* (Diptera: Ceratopogonidae) to oral infection with bluetongue virus. Archiv. Virol. 95: 177–182.

Jones, R. H., and N. M. Foster. 1971a. Transovarian transmission of bluetongue virus unlikely for *Culicoides variipennis.* Mosq. News 31: 434–437.

Jones, R. H., and N. M. Foster. 1971b. The effect of repeated bloodmeals infective for bluetongue on the infection rate of *Culicoides variipennis.* J. Med. Entomol. 8: 499–501.

Jones, R. H., and N. M. Foster. 1974. Oral infection of *Culicoides variipennis* with bluetongue virus: development of susceptible and resistant lines from a colony population. J. Med. Entomol. 11: 316–323.

Jones, R. H., and N. M. Foster. 1978a. Heterogeneity of *Culicoides variipennis* field populations to oral infection with bluetongue virus. Am. J. Trop. Med. Hyg. 27: 178–183.

Jones, R. H., and N. M. Foster. 1978b. Relevance of laboratory colonies of the vector in arbovirus research - *Culicoides variipennis* and bluetongue. Am. J. Trop. Med. Hyg. 27: 168–177.

Jones, R. H., A. J. Luedke, T. E. Walton, and H. E. Metcalf. 1981. Bluetongue in the United States: an entomological perspective toward control. World Anim. Rev. 38: 2–8.

Kay, B. H., I. D. Fanning, and P. Mottram. 1989. Rearing temperature influences flavivirus vector competence of mosquitoes. Med. Vet. Entomol. 3: 415–422.

Linhares, A. X., and J. R. Anderson. 1990. The influence of temperature and moonlight on flight activity of *Culicoides variipennis* (Coquillett) (Diptera: Ceratopogonidae) in northern California. Pan-Pac. Entomol. 66: 199–207.

Loomis, E. C., R. B. Bushnell, and B. I. Osburn. 1985. Epidemiologic study of bluetongue virus on the Tejon Ranch, California: Vector, host, virus relationships. Prog. Clin. Biol. Res. 178: 1–9.

Luedke, A. J., R. H. Jones, and M. M. Jochim. 1967. Transmission of bluetongue between sheep and cattle by *Culicoides variipennis.* Am. J. Vet. Res. 28: 457–460.

Mecham, J. O., V. C. Dean, J. G. Wigington, and R. A. Nunamaker. 1990. Detection of bluetongue virus in *Culicoides variipennis* (Diptera: Ceratopogonidae) by an antigen capture enzyme-linked immunosorbent assay. J. Med. Entomol. 27: 602–606.

Mellor, P. S. 1990. The replication of bluetongue virus in *Culicoides* vectors. Curr. Top. Microbiol. Immunol. 162: 143–161.

Mellor, P. S., P. Rawlings, M. Baylis, and M. P. Wellby. 1998. Effect of temperature on African horse sickness virus infection in *Culicoides.* Archiv. Virol. 14 (Suppl.): 155–163.

Metcalf, H. E., J. E. Pearson, and A. L. Klingsporn. 1981. Bluetongue in cattle: a serologic survey of slaughter cattle in the United States. Am. J. Vet. Res. 42: 1057–1061.

Minitab. 1996. Minitab reference manual, release 11. Minitab, State College, PA.

Mullens, B. A. 1985. Age-related adult activity and sugar feeding by *Culicoides variipennis* (Diptera: Ceratopogonidae) in southern California, USA. J. Med. Entomol. 22: 32–37.

Mullens, B. A. 1989. A quantitative survey of *Culicoides variipennis* (Diptera: Ceratopogonidae) in dairy wastewater ponds in southern California. J. Med. Entomol. 26: 559–565.

Mullens, B. A. 1995. Flight activity and response to carbon dioxide of *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) in southern California. J. Med. Entomol. 32: 310–315.

Mullens, B. A., and A. C. Gerry. 1998. Comparison of bait cattle and carbon dioxide-baited suction traps for collecting *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) and *Culex quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 35: 245–250.

Mullens, B. A., and F. R. Holbrook. 1991. Temperature effects on the gonotrophic cycle of *Culicoides variipennis* (Diptera: Ceratopogonidae). J. Am. Mosq. Control Assoc. 7: 588–591.

Mullens, B. A., W. J. Tabachnick, F. R. Holbrook, and L. H. Thompson. 1995. Effects of temperature on virogenesis of bluetongue virus serotype 11 in *Culicoides variipennis sonorensis.* Med. Vet. Entomol. 9: 71–76.

Nelson, R. L., and R. E. Bellamy. 1971. Patterns of flight activity of *Culicoides variipennis* (Coquillett) (Diptera: Ceratopogonidae). J. Med. Entomol. 8: 283–291.

Nevill E. M., B. J. Erasmus, and G. J. Venter. 1991. A six-year survey of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae), pp. 314–319. *In* T. E. Walton and B. I. Osburn [eds.], Bluetongue, African horse sickness, and related orbiviruses. CRC, Boca Raton, FL.

Nunamaker, R. A., P. J. Sieburth, V. C. Dean, J. G. Wigington, C. E. Nunamaker, and J. O. Mecham. 1990. Absence of transovarial transmission of bluetongue virus in *Culicoides variipennis:* immunogold labeling of bluetongue virus antigen in developing oocytes from *Culicoides variipennis* (Coquillett). Comp. Biochem. Physiol. A. 96: 19–31.

Nunamaker, R. A., J. O. Mecham, F. R. Holbrook, and J. A. Lockwood. 1997. Applications of Dot-Blot, ELISA, and immunoelectron microscopy to field detection of bluetongue virus in *Culicoides variipennis sonorensis:* an ecological perspective. J. Med. Entomol. 34: 24–28.

Osburn, B. I., B. McGowan, B. Heron, E. Loomis, R. Bushnell, M. Stott, and W. Utterback. 1981. Epizootiologic study of bluetongue: virologic and serologic results. Am. J. Vet. Res. 42: 884–887.

Potter, H. W., and D. H. Akey. 1978. Pigmentation associated with oogenesis in the biting midge, *Culicoides variipennis:* changes in abdominal tergite patterns. Mosq. News 38: 499–504.

Reisen, W. K. 1989. Estimation of vectorial capacity: Introduction. Bull. Soc. Vector Ecol. 14: 39–40.

Reisen, W. K., H. D. Lothrop, and J. L. Hardy. 1995. Bionomics of *Culex tarsalis* (Diptera: Culicidae) in relation to arbovirus transmission in southeastern California. J. Med. Entomol. 32: 316–327.

Roberts, R. H. 1972. Relative attractiveness of CO_2 and a steer to Tabanidae, Culicidae, and *Stomoxys calcitrans.* Mosq. News 32: 208–211.

Rosenberg, R., R. G. Andre, and L. Somchit. 1990. Highly efficient dry season transmission of malaria in Thailand. Trans. R. Soc. Trop. Med. Hyg. 84: 22–28.

Rossitto, P. V., and N. J. MacLachlan. 1992. Neutralizing epitopes of the serotypes of bluetongue virus present in the United States. J. Gen. Virol. 73: 1947–1952.

Sellers, R. F., and P. S. Mellor. 1993. Temperature and the persistence of viruses in *Culicoides* spp. during adverse conditions. Rev. Sci. Tech. Off. Int. Epiz. 12: 733–755.

Singer, R. S., W. M. Boyce, I. A. Gardner, W. O. Johnson, and A. S. Fisher. 1998. Evaluation of bluetongue virus diagnostic tests in free-ranging bighorn sheep. Prev. Vet. Med. 35: 265–282.

Standfast, H. A., A. L. Dyce, and M. J. Muller. 1985. Vectors of bluetongue virus in Australia. Prog. Clin. Biol. Res. 178: 177–186.

Stott, J. L., B. I. Osburn, R. Bushnell, E. C. Loomis, and K.R.E. Squire. 1985. Epizootiological study of bluetongue virus infection in California livestock: an overview. Prog. Clin. Biol. Res. 178: 571–582.

Tabachnick, W. J. 1991. Genetic control of oral susceptibility to infection of *Culicoides variipennis* with bluetongue virus. Am. J. Trop. Med. Hyg. 45: 666–671.

Tabachnick, W. J. 1996. *Culicoides variipennis* and bluetongue-virus epidemiology in the United States. Annu. Rev. Entomol. 41: 23–43.

Thomas, F. C. 1985. Plaque neutralization cross reactivity of post infection bovine sera amongst bluetongue virus serotypes. Prog. Clin. Biol. Res. 178: 511–512.

Turell, M. J. 1993. Effect of environmental temperature on the vector competence of *Aedes taeniorhynchus* for Rift Valley fever and Venezuelan equine encephalitis viruses. Am. J. Trop. Med. Hyg. 49: 672–676.

Ward, M. P. 1996. Climatic factors associated with the infection of herds of cattle with bluetongue viruses. Vet. Res. Com. 20: 273–283.

Ward, M. P., and M. C. Thurmond. 1995. Climatic factors associated with risk of seroconversion of cattle to bluetongue viruses in Queensland. Prev. Vet. Med. 24: 129–136.

Wieser-Schimpf, L., W. C. Wilson, D. D. French, A. Baham, and L. D. Foil. 1993. Bluetongue virus in sheep and cattle and *Culicoides variipennis* and *C. stellifer* (Diptera: Ceratopogonidae) in Louisiana. J. Med. Entomol. 30: 719–724.

Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232–237.

Wright, J. C., R. R. Getz, T. A. Powe, K. E. Nusbaum, D. A. Stringfellow, G. R. Mullen, and L. H. Lauerman. 1993. Model based on weather variables to predict seroconversion to bluetongue virus in Alabama cattle. Prev. Vet. Med. 16: 271–278.

Received for publication 30 November 1999; accepted 5 October 2000.