

Environmental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpreting laboratory data in a field context

B.A. Mullens⁽¹⁾, A.C. Gerry⁽¹⁾, T.J. Lysyk⁽²⁾ & E.T. Schmidtman⁽³⁾

(1) Department of Entomology, University of California, Riverside, CA 92521, United States of America

(2) Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, Alberta T1J 4B1, Canada

(3) USDA-ARS, Arthropod-Borne Animal Diseases Research Laboratory, P.O. Box 3965, University Station, Laramie, WY 82071, United States of America

Summary

Environmental factors profoundly affect vectorial capacity, governing dynamics and intensity of vector-vertebrate contact in time and space (e.g. seasonal vector population densities, biting rates, and feeding frequencies). Temperature influences vector developmental rates and life history parameters, and may modify vector competence. Studies should move iteratively from field to laboratory, as attempts are made to understand complex epidemiological patterns. Simulation models can be extremely helpful in identifying and predicting geographic and seasonal trends in virus occurrence. Field and laboratory data from the *Culicoides sonorensis*-bluetongue virus system in North America are incorporated into preliminary estimates of virus prevalence and geographic occurrence along a latitudinal (and temperature) gradient. Geographic information systems technology is likely to be helpful in understanding vector and virus occurrence on a broader scale, especially in temperate latitudes that typify sporadic or emerging transmission zones, areas of particular concern for animal movement.

Keywords

Bluetongue – *Culicoides* – Geographic information systems – Modelling – Temperature – United States of America – Vector competence – Vectorial capacity – Virogenesis.

Since 1998, bluetongue (BT) viruses (BTV) have caused unprecedented levels of sheep disease and associated losses in historically uninfected areas of the Mediterranean Basin (14). This serious outbreak has renewed interest in epidemiology. The viruses are transmitted biologically by small blood-feeding midges in the genus *Culicoides* (13, 22). Key Old World vectors include the subgenus *Avaritia*, particularly *C. imicola*. In North America, the principal vector is *C. sonorensis* (formerly *C. variipennis*) (11), while in Central America and the Caribbean other species, such as *C. insignis*, appear to be responsible (23). Thus, there are at least three different *Culicoides* subgenera and several species already implicated as BTV vectors, and there are probably more. In combination with the 24 known virus serotypes, the diversity of vectors and ruminant hosts would be expected to yield measurable differences in epidemiology and transmission

dynamics in ecologically different regions. It is remarkable that, despite this high diversity, seasonal BTV transmission patterns are frequently similar even on different continents.

While BTV is widely established in tropical zones, our understanding of its epidemiology (with a few exceptions) is incomplete. Understanding epidemiology in tropical endemic zones would probably be very helpful in interpreting seasonal transmission patterns in subtropical or temperate zones; this area deserves more study. Sustained efforts and published field work have been conducted in subtropical and temperate areas of South Africa, Israel, Australia, and western North America. In-depth field studies also have been done in parts of southern Europe (particularly the Iberian Peninsula), Morocco, and the Caribbean Basin. Sites chosen for study share some important

characteristics. The subtropical/temperate sites do not have significant year-round virus transmission, as far as has been demonstrated, and are positioned on a cline (usually basically north-south) that incorporates a continuum of virus transmission intensity. Many sites of concern have a Mediterranean-type climate, with cooler, wetter winters and hotter, drier summers. All the work cannot be reviewed here, but understanding environmental driving mechanisms should help in the understanding, and hopefully the prediction of BTV transmission in a variety of circumstances.

Vector competence and vectorial capacity

These terms should be clarified. Vector competence refers to the ability of a vector to support virus infection and replication and/or dissemination. It can apply to a single insect, although hopefully populations can be described. Estimators, such as infection rate, typically do not include transmission, but true competence should include ability to transmit. Experimental control and expedience result in vector competence being measured in the laboratory, with results extrapolated to field settings. Vectorial capacity, on the other hand, is the more inclusive ecological parameter and refers to the ability of the vector population to transmit a pathogen. It incorporates biting rates, survivorship, and extrinsic incubation period, and all of these are subject to environmental modification. To have any real meaning, at least the first two of these parameters must be measured in the field. Vectorial capacity may also include competence, which serves as an arithmetic correction factor in the numerator of the equation below. Vectorial capacity is described using:

$$C = ma^2Vp^n / -\log p$$

where:

C = vectorial capacity (transmissions per active case per day)

ma = bites per host per day

a = host preference/length of time between blood meals

V = vector competence

p = daily survival probability of the vector

n = extrinsic incubation period.

Vectorial capacity is more likely to be useful in measuring changes in the ability of a vector population to transmit, rather than accurately estimating transmission (e.g. cases/day) (6).

There is a critical difference between capacity and competence. A 'competent' vector may have a low vectorial capacity due to low biting rates or survivorship, while a vector with low competence may be important. For example, *C. brevitarsis* in Australia has low competence but serves as a vector due to high biting rates, while *C. fulvus* is quite competent but has low vectorial capacity (at least to sheep) through abundance and geographic distribution (21). 'Vector competence' often is used when 'vectorial capacity' is intended. A laboratory demonstration of vector competence is not the only necessary step to implicate a vector(s).

In this paper, the authors will make a plea to examine environmental factors affecting vectorial capacity in the field and *in vivo* whenever possible.

Environment and transmission

Environmental influences on vectorial capacity can be assessed on several levels. Examining the historical record can be powerful and exciting, provided the data are sufficiently complete. Historical data usually lack detailed vector information, indirectly reflect vectorial capacity, and thus should be viewed differently from experimental studies. For example, the *Culicoides*-transmitted African horse sickness virus (AHSV) causes severe equine mortality and generated a useable data set (AHS-induced horse mortality and weather records) stretching back nearly two centuries. This allowed Baylis *et al.* (1) to examine the effect of the El Niño/southern oscillation index on disease occurrence, and thereby indirectly gain some insights into vectorial capacity as it fluctuated with rainfall patterns. Obviously, it is not possible in such a case to ascertain exactly how the rainfall patterns were related to vectorial capacity, but that can be studied later. Other modelling efforts couple more current data with historical data (4, 5). The Braverman *et al.* (4) study was unusual in that 30 years of BTV data for Israel, plus several years of vector population data, were accessible. Modelling efforts and improved environmental descriptive tools including geographic information systems technology have considerable promise in demonstrating 'big picture' trends and highlighting environmental factors that should be examined more closely.

Temperature effects

Many factors affect vectorial capacity, but none is more potent or predictable than temperature. Temperature is a driving force for immature vector developmental rates. This influences the number of generations produced and the adult population size that can result in a season. Rearing temperature influences adult size (and may be related to

survivorship and competence), and ambient temperature affects adult survivorship. Temperature governs gonotrophic cycle length and thus adult feeding frequency and the opportunity to acquire or transmit BTV. In the adult midge body, temperature influences virus infection/replication. The relatively robust mosquito-arbovirus literature deserves examination as attempts are made to understand *Culicoides*-virus interactions. After a 'competent' adult *Culicoides* female feeds on a BTV-infected ruminant host, the process is similar to most arbovirus-vector systems (7, 10, 13). It can fail at many points, resulting in an 'incompetent' midge. In the mesenteron lumen, virus must penetrate the mid-gut cells, or bypass them via the 'leaky gut' phenomenon to enter the haemocoel directly. This mid-gut infection barrier is a common point at which infection fails. If BTV penetrates the mid-gut cells, it must also later escape to the haemocoel (mid-gut escape barrier), disseminate and replicate (dissemination barrier). The virus then must invade and then escape the salivary glands during the act of blood-feeding. Infection will not be discussed in detail, but some aspects relate to environmental modification.

Adult midge competence is genetically determined (22), but environmentally influenced. Environmental influences probably begin in immature habitats; this deserves more research attention. Larval mosquitoes stressed by nutrition or high temperatures emerge as smaller adults, which may be more susceptible to arbovirus infection, although survivorship also may be compromised (10). *Culicoides* size in the field varies inversely with temperature and nutrition (17), although neither has been specifically related to BTV competence. In the laboratory, *C. nubeculosus*, which ordinarily has very low competence, becomes a moderately competent vector for AHSV (10% infection rate) when reared at very high temperatures, and similar laboratory data exist for BTV (13, 24).

Mid-gut infection and escape barriers are probably common in the *Culicoides*-BTV system. Individual *C. sonorensis* refractory to virus infection did harbour low levels of mid-gut virus (7). Gerry *et al.* (9) fed field-collected nulliparous midges on blood spiked with a local (southern California) strain of BTV-10. Although 80% of these flies did not reach ELISA readings for 'infection', they were higher than non-virus-fed controls, implying virus presence. There is some evidence for a dissemination barrier in *C. sonorensis*, but little evidence that salivary gland infection or escape barriers exist (7). However, insights into the BTV infection process are based almost entirely on work with a single vector species amenable to manipulation – *C. sonorensis*. While

difficult, it is not impossible to conduct experimental work using field-collected *Culicoides*, such as the subgenus *Avaritia*; this aspect deserves more attention. This is easier if appropriate laboratory facilities are close to field sites, and getting the insects to behave and survive can be a challenge. Using field-collected midges for infection and even transmission studies has been successful, especially in South Africa and Australia (3, 19).

The effect of temperature on bluetongue virus competence

Because virogenesis is faster at higher temperatures (18, 19, 24), the appropriate extrinsic incubation period varies dramatically. For example, 4 to 8 days may allow virus titres to peak at constant temperatures of 27–32°C, while at 21°C the midges might require 16 to 22 days. Overall, mostly using *C. sonorensis*, laboratory studies suggest that ultimate BTV infection rates may not vary significantly with temperature, provided they are above minimum threshold temperatures for virus replication (approximately 9–15°C, although this deserves more study). Latent virus may persist, perhaps at levels that are not easy to detect at low temperatures, and then replicate rapidly when midges experience higher temperatures. However, differential temperature effects on virogenesis among vector species are possible (19). Gerry *et al.* (9) showed that infection rates of field-collected *C. sonorensis* fed BTV-10 in the laboratory were similar across the season and over multiple years, a pattern also shown in South Africa with a different set of vectors and BT viruses (19). Thus, if immature developmental temperatures do affect subsequent adult competence for BTV, it has not been evident at least over the several months in summer-autumn when vector populations are high.

There are difficulties in such assessments. Firstly, the measurement of 'infection' should be clarified. 'Infected' vectors will be portrayed as 'infective' ones that can transmit virus. Assessments seldom include actual shedding of virus in saliva, much less determine whether the bite might transmit sufficient virus to infect a ruminant. Rather, measurements of infection are usually related to recoverable live virus from the ground-up insect body via embryonated eggs or tissue culture, or perhaps do not involve live virus at all (e.g. ELISA, and definitely PCR). Further studies of the relationship between current measures of infection and actual transmission are needed. Ideally, this would be via bites of individual insects to naive ruminants – a very significant and expensive effort to be sure, but not beyond our reach. Secondly, just as virogenesis is dramatically affected by temperature, so is survivorship. Higher

temperatures decrease extrinsic incubation period and gonotrophic cycle length and positively affect vectorial capacity. However, high temperatures decrease daily survivorship and exert a negative effect. In the vectorial capacity equation, survivorship is raised to the power of n (duration of extrinsic incubation in days). Survivorship and extrinsic incubation are extremely potent modifiers of capacity; laboratory information on temperature-dependent virogenesis and competence thus must be coupled with data on temperature versus gonotrophic cycle length and field survivorship. Only then can the probable impact of temperature on transmission locally or over a larger area be understood.

Environment and the bluetongue virus-*Culicoides sonorensis* system

The graphic of Metcalf *et al.* (15) shows a cline in BTV transmission, albeit indirectly through cattle serology. High virus transmission in the south-western United States of America (USA) declines as one moves east and north. There is very little BTV activity in Canada, but considerable interest in determining the circumstances under which it might occur, and thus in delineating BTV-free zones. Data from North America and elsewhere are being incorporated in simulation efforts in Canada, and additional work is being conducted, with emphasis on temperature effects.

Assessing environmental conditions and their relationship to the transmission of arthropod-borne pathogens is daunting, particularly on scales appropriate to insect populations or species. Ongoing United States Department of Agriculture efforts document distribution of the vector *C. sonorensis* and its sister species, *C. variipennis* (which does not appear to support BTV infection) in the north-central USA, and are developing geographic information systems (GIS) as a predictive tool in this transition zone both for virus activity and the two *Culicoides* species. These GIS methods provide robust opportunities for processing, analysing, and displaying spatially-based environmental data concerning vector-borne diseases (12). GIS analyses also facilitate interpretation of spatially-related environmental factors, including climate, that influence vector population presence and abundance (2). In North America, GIS methods are being used to examine the association between environmental factors and *C. sonorensis* distribution. The analysis is based on the presence and absence of *C. sonorensis* on cattle ranches across North Dakota, South Dakota,

and Nebraska (data developed as part of a recent BTV epidemiology study reported elsewhere at this Symposium). Surface soil salinity is a promising factor, influencing distribution of *C. sonorensis*; levels of dissolved salts differ in aquatic habitats occupied by respective species of the *C. variipennis* complex (20). Along with other attributes and continuous variable data, soil salinity between positive and negative sites at ranch and regional levels should provide a better understanding of *C. sonorensis* distribution. GIS methods can then be used to predict other areas where *C. sonorensis* should not occur, and thus will be useful as support for regionalised export of livestock from the USA. GIS can be used to examine environmental effects on vectorial capacity, as expressed through vector population dynamics, adult survival, extrinsic incubation period, etc. For example, in North Dakota *C. sonorensis* is widespread west of the Missouri River, but BTV transmission is rare or absent. This relationship may be a function of temperature effects on virogenesis and the extrinsic incubation period. However, other environmental factors also need to be considered, since these populations exist at the margin of the species' range and therefore may be subject to less typical or unique environmental conditions.

A simulation exercise determined if the latitudinal gradient of BT incidence in North America could be related to temperature-mediated components of the vectorial capacity equation from laboratory studies. The relationship between temperature and virogenesis rate in the vector (Fig. 1), is a curvilinear function of the form:

$$rEI(T) = 1/\text{Time} = 0.0003 \cdot T \cdot (T - 10.4057) \text{ where:}$$

Time = the duration of the extrinsic incubation period in days

T = temperature (°C). The relationship between temperature and ovarian development rate (16), and hence feeding, can be described as:

$$rOD(T) = 0.000171 \cdot T \cdot (T - 3.6966) \cdot (41.8699 - T)^{1/2.7056} \text{ (Fig. 1).}$$

Data on relationships between vector survival and temperature from the field do not lend themselves well to development of models over a broad geographic scale, since they may be site-specific. Still, strong seasonal trends in survival (far higher in winter compared to summer) are evident and important in interpreting transmission dynamics and addressing such questions as overwintering (8).

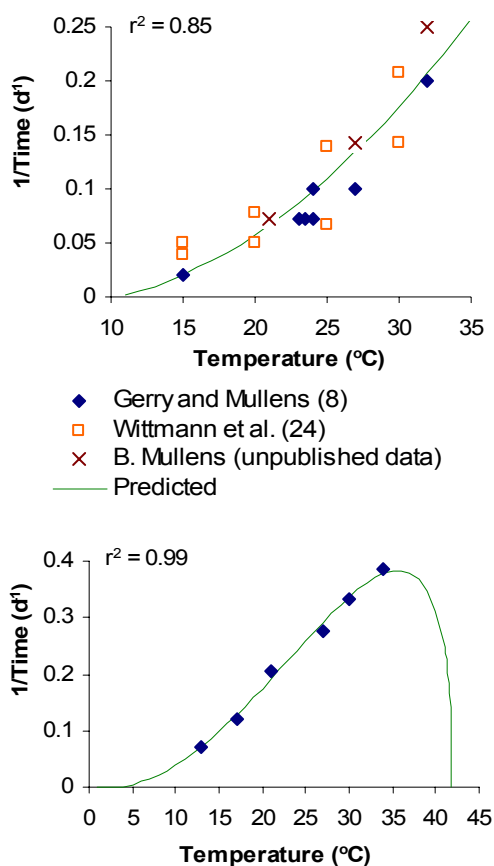


Figure 1
Development rate (1/extrinsic incubation period in days) of bluetongue virus (top panel) and the first gonotrophic cycle (1/time to oviposition) (bottom panel) in *C. sonorensis* as a function of temperature (x-axis)

Daily maximum-minimum temperatures for August to December 1977 were obtained for 16 weather stations representing a latitudinal gradient from California to North Dakota to correspond with reported data on the prevalence of BTV infection in Arizona, California, Colorado, Montana, North Dakota, New Mexico, Utah and Wyoming. Monthly mean temperatures were calculated for August and September, and show a distinct cline with latitude (Fig. 2). Temperatures were expected to decline with latitude, but the change was surprisingly large.

The daily maxima-minima for each site were used to generate hourly temperatures to account for diurnal variation in temperature. A rate summation model then determined the length of the extrinsic incubation period (EI) and number of ovarian cycles required to transmit BT, assuming vectors obtained an infective blood meal on 1 August. For ovarian development, time for second and subsequent cycles was estimated as 70% of the first cycle duration. The existence of a latitudinal cline in the EI is clear, and hence the number of ovarian cycles required for BTV transmission (Fig. 3). The EI increases from

about 5 d to >20 d across latitude, and the number of cycles required for transmission also increases. At lower latitudes, from 2 to 3 cycles are required for transmission, while >4 cycles are required at higher latitudes. These trends were correlated with the reported prevalence of BTV infection. No significant transmission occurred when the simulated EI was >17 d. High levels of BTV infection were observed when 2 to 3 cycles only were required for transmission, and lower levels observed when 4 to 5 cycles were required. The exception is a single observation of 0.1 incidence when the number of cycles required = 4.

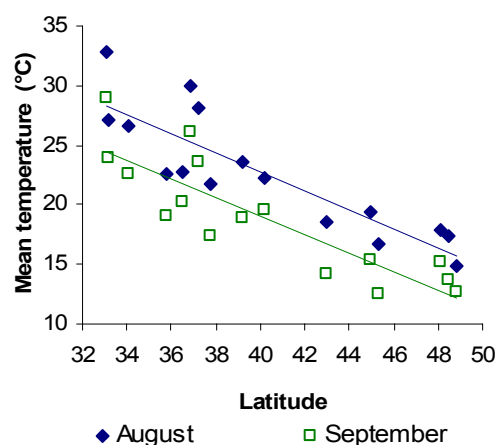


Figure 2
Changes in mean monthly temperatures (y-axis) with latitude for August and September 1977 in western North America

These preliminary results will be expanded using data currently being collected. The results illustrate that environmental factors can influence vectorial capacity, but relationships need to be refined. A major gap in current knowledge of *Culicoides* spp. is detailed information on where they rest, and what the temperatures, or other factors, such as humidity, are in these microenvironments. Resting insects may be assumed to be in areas sheltered from temperature extremes. However, calculating degree days or degree hours necessary for oogenesis or virus development requires better resolution than merely using mean daily air temperatures. This is especially true in cool weather, when daily means would ignore periods of perhaps several hours each day when virogenesis could occur. Adult *Culicoides* are only detected when they are flying. Usually they are seeking a blood-meal, but may be seeking oviposition sites, or going to or from a resting site. However, *Culicoides* spend well over 90% of their time resting (e.g. developing the oocytes to the appropriate stage for acquiring a blood-meal, digesting the blood-meal and developing eggs). Understanding the thermal ecology of the vectors

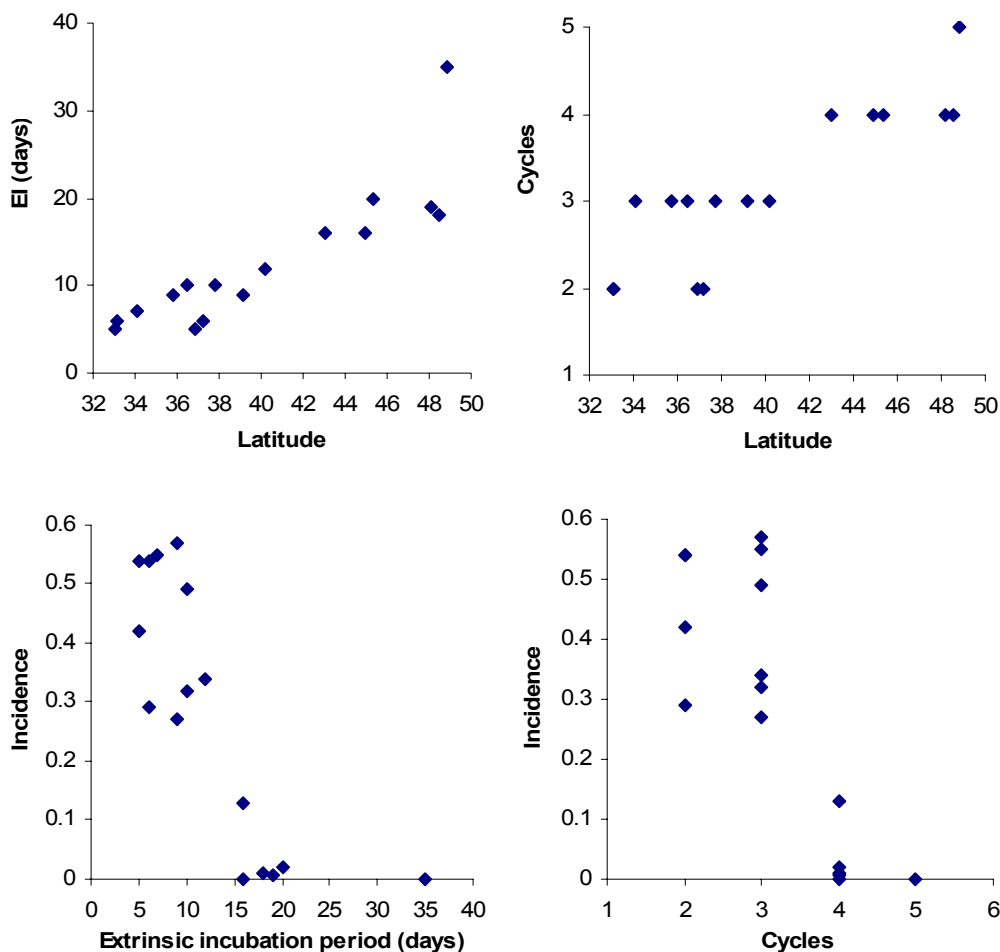


Figure 3
Changes with latitude in the estimated extrinsic incubation period (y-axis) of bluetongue virus in days (upper left) and number of ovarian cycles (y-axis) required for transmission of bluetongue virus (upper right)
Reported prevalence of BTV (15) in relation to simulated EI (lower left) and ovarian cycles required for transmission (lower right)

and the viruses they may harbour is a critical aspect of understanding the possible role of the vector in BTV overwintering, and this deserves much more field and laboratory study.

References

1. Baylis M., Mellor P.S. & Meiswinkel R. (1999). – Horse sickness and ENSO in South Africa. *Nature*, **397**, 574.
2. Baylis M., Mellor P.S., Wittmann E.J. & Rogers D. (2001). – Prediction of areas around the Mediterranean at risk of bluetongue by modelling the distribution of its vector using satellite imaging. *Vet. Rec.*, **149**, 639-643.
3. Bellis G., Gibson D., Polkinghorne I., Johnson S. & Flanagan M. (1994). – Infection of *Culicoides brevitarsis* and *C. wadai* (Diptera: Ceratopogonidae) with 4 Australian serotypes of bluetongue virus. *J. Med. Entomol.*, **31**, 382-387.
4. Braverman Y., Chechik F. & Mullens B. (2001). – The interaction between climatic factors and bluetongue outbreaks in Israel and the eastern Mediterranean, and the feasibility of establishing bluetongue-free zones. *Israel J. Vet. Med.*, **56**, 99-109.
5. Capela R., Purse B., Pena I., Wittmann E.J., Margarita Y., Capela M., Romao L., Mellor P.S. & Baylis M. (2003). – Spatial distribution of *Culicoides* species in Portugal in relation to the transmission of African horse sickness and bluetongue viruses. *Med. Vet. Entomol.*, **17**, 165-177.
6. Dye C. (1992). – The analysis of parasite transmission by bloodsucking insects. *Ann. Rev. Entomol.*, **37**, 1-19.
7. Fu H., Leake C., Mertens P.P.C. & Mellor P.S. (1999). – The barriers to bluetongue infection, dissemination and transmission in the vector, *Culicoides variipennis* (Diptera: Ceratopogonidae). *Arch. Virol.*, **144**, 747-761.
8. Gerry A. & Mullens B. (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.
9. Gerry A., Mullens B., MacLachlan N.J. & Mecham J. (2001). – Seasonal transmission of bluetongue virus by *Culicoides sonorensis* (Diptera: Ceratopogonidae) on a southern California dairy and evaluation of vectorial capacity as a predictor of virus transmission. *J. Med. Entomol.*, **38**, 197-209.
10. Hardy J. (1988). – Susceptibility and resistance of vector mosquitoes. In *The arboviruses: epidemiology and ecology* (T. Monath, ed). CRC Press, Boca Raton, 87-126.
11. Holbrook F., Tabachnick W., Schmidtman E., McKinnon C., Bobian R. & Grogan W. (2000). – Sympatry in the *Culicoides variipennis* complex: a taxonomic reassessment. *J. Med. Entomol.*, **37**, 65-76.
12. Kitron U. (1998). – Landscape ecology and epidemiology of vector-borne diseases – tools for spatial analysis. *J. Med. Entomol.*, **35**, 435-445.
13. Mellor P., Boorman J. & Baylis M. (2000). – *Culicoides* biting midges: their role as arbovirus vectors. *Ann. Rev. Entomol.*, **45**, 307-340.
14. Mellor P.S. & Wittmann E.J. (2002). – Bluetongue virus in the Mediterranean Basin 1998-2001. *Vet. J.*, **164**, 20-37.
15. Metcalf H., Pearson J. & Klingsporn A. (1981). – Bluetongue in cattle: a serological survey of slaughter cattle in the United States. *Am. J. Vet. Res.*, **42**, 1057-1061.
16. Mullens B. & Holbrook F. (1991). – Temperature effects on the gonotrophic cycle of *Culicoides variipennis* (Diptera: Ceratopogonidae). *J. Am. Mosquito Control Assoc.*, **7**, 588-591.
17. Mullens B. & Rodriguez J. (1988). – Colonization and response of *Culicoides variipennis* (Diptera: Ceratopogonidae) to pollution levels in experimental dairy wastewater ponds. *J. Med. Entomol.*, **25**, 441-451.
18. Mullens B., Tabachnick T., Holbrook F. & Thompson L. (1995). – Effects of temperature on virogenesis of bluetongue serotype 11 in *Culicoides variipennis sonorensis*. *Med. Vet. Entomol.*, **9**, 71-76.
19. Paweska J., Venter G. & Mellor P. (2002). – Vector competence of South African *Culicoides* species for bluetongue serotype 1 (BTV-1) with special reference to the effect of temperature on the rate of virus replication in *C. imicola* and *C. bolitinos*. *Med. Vet. Entomol.*, **16**, 10-21.
20. Schmidtman E., Bobian R. & Belden R. (2000). – Soil chemistries define aquatic habitats with immature populations of the *Culicoides variipennis* complex (Diptera: Ceratopogonidae). *J. Med. Entomol.*, **37**, 58-64.
21. Standfast H., Dyce A. & Muller M. (1985). – Vectors of bluetongue virus in Australia. *Prog. Clin. Biol. Res.*, **181**, 177-186.
22. Tabachnick W. (1996). – *Culicoides variipennis* and bluetongue virus epidemiology in the United States. *Ann. Rev. Entomol.*, **41**, 23-43.
23. Tanya V., Greiner E. & Gibbs, E. (1992). – Evaluation of *Culicoides insignis* (Diptera: Ceratopogonidae) as a vector of bluetongue virus. *Vet. Microbiol.*, **32**, 1-14.
24. Wittmann E.J., Mellor P.S. & Baylis M. (2002). – Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. *Med. Vet. Entomol.*, **16**, 147-156.