# Overwintering of Bluetongue virus in temperate zones

Christie E. Mayo[1*], Bradley A. Mullens[2], E. Paul J. Gibbs[3] and N. James MacLachlan[4]

[1] Department of Microbiology, Immunology, and Pathology, College of Veterinary and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA.
[2] Department of Entomology, University of California, Riverside, CA, USA.
[3] College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.
[4] Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA, USA.

[*] Corresponding author at: Department of Microbiology, Immunology, and Pathology, College of Veterinary and Biomedical Sciences, Colorado State University, 300 West Drake Road, Fort Collins, CO 80526, USA.
Tel.: +1-970-297-1287, e-mail: Christie.Mayo@colostate.edu.

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Arbovirus transmission,
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*Culicoides sonorensis*,
Interseasonal maintenance,
Virus overwintering.

### Summary
Within Northern California, *Culicoides sonorensis* is the major vector of Bluetongue virus (BTV) and annual infection of livestock is distinctly seasonal (typically July-November). Our recent studies compare the population dynamics of *C. sonorensis* midges with occurrence of BTV infection of *C. sonorensis* and sentinel dairy cattle throughout both the seasonal and interseasonal ('overwintering') periods of BTV activity. Spring emergence and seasonal abundance of adult *C. sonorensis* on the sampled farms coincided with rising vernal temperature. Intensive surveillance confirmed widespread infection of both sentinel cattle and vector midges during the August-November period of seasonal BTV transmission. Bluetongue virus infection of parous female midges captured in traps set during daylight hours was also detected during the interseasonal period of virus activity, whereas there was no concurrent active infection of sentinel cattle during the overwintering period. The finding of BTV-infected vector midges during mid-Winter suggests that BTV can overwinter in Northern California by infection of long-lived female *C. sonorensis* midges that were infected during the prior seasonal period of virus transmission and which, then, entered a quiescence in the fall (Autumn) and re-emerged sporadically during the overwintering period. Notably, vertical transmission of BTV was not detected among progeny of midges infected in the laboratory nor in field-collected larvae. In addition to defining the mechanism of BTV over-wintering in a temperate region, the studies reviewed in this article also provide precise documentation of temporal changes in the annual abundance, dispersal and dynamics of BTV infection of *Culicoides* midges. Collectively these findings are critical to the creation of accurate predictive models of BTV infection in livestock and to development of sound abatement strategies.

## Svernamento del virus della Bluetongue in zone temperate

### Parole chiave
Animale sentinella,
Arbovirus,
Bluetongue,
California,
*Culicoides sonorensis,*
Svernamento virale,
Virus della Bluetongue.

### Riassunto
*Culicoides sonorensis* è il principale vettore del virus Bluetongue (BTV) nel Nord della California dove la Bluetongue è una malattia stagionale (in genere da luglio a novembre). In un nostro recente studio si sono confrontate le dinamiche di popolazione di *C. sonorensis* con la presenza di BTV nei *C. sonorensis* e in bovini da latte sentinella durante i periodi stagionali e interstagionali di attività del virus (*overwintering*). La comparsa in primavera e l'abbondanza stagionale di *C. sonorensis* allo stadio adulto nelle aziende esaminate hanno coinciso con l'aumento delle temperature. La sorveglianza intensiva ha confermato l'infezione estesa sia negli animali sentinella che nei vettori nel periodo di trasmissione stagionale del virus, da agosto a novembre. Il rilevamento di *Culicoides* parous infetti, catturati nelle ore diurne durante il periodo interstagionale dell'attività virale in assenza di sierconversioni negli animali sentinella, suggerisce che nel Nord della California il virus possa sopravvivere alla stagione sfavorevole attraverso le femmine adulte di *C. sonorensis* infettate durante la stagione vettoriale che vanno in diapausa durante l'autunno per tornare quindi attive in primavera. È importante inoltre rilevare come non sia stata riscontrata la trasmissione verticale di BTV nella progenie di *Culicoides* infettate in laboratorio né nelle larve raccolte in campo. Oltre

a definire i possibili meccanismi attraverso cui BTV sopravvive alla stagione sfavorevole in una regione temperata, lo studio fornisce una precisa documentazione sui cambiamenti di abbondanza e dispersione e sulle dinamiche annuali dell'infezione da BTV del *C. sonorensis*. Complessivamente questi risultati permettono di definire un modello predittivo dell'infezione da BTV nel bestiame da latte e di stabilire strategie efficaci per ridurre la diffusione del virus.

## Introduction

Bluetongue virus (BTV) infection of animals at temperate latitudes is distinctly seasonal (typically July-November in the Northern Hemisphere). However, it remains uncertain how BTV persists during winter months, when cold temperatures restrict vector activity and reproduction (Mayo *et al.* 2012, Nevill 1971, Wilson *et al.* 2008). The mechanism of this so-called 'overwintering' of BTV in endemic temperate regions has vexed scientists since the original published descriptions of BT in South Africa over a century ago (Spreull 1905). Even the term 'overwintering' is somewhat misleading, because the interseasonal period of transmission cessation typically is greater than 6 months in temperate regions (Erasmus 1975, MacLachlan and Mayo 2013, Nevill 1971, Spreull 1905, Stott 1985). In this review we summarize findings from our recent intensive field and laboratory studies to better characterize potential mechanisms of BTV overwintering within temperate zones, namely in Northern California, where BTV is seasonally endemic in livestock and *Culicoides sonorensis* serves as the primary vector of the virus (Mayo *et al.* 2012, Mayo *et al.* 2014 a, b, Osborne *et al.* 2015). These studies have included:

1. intensive surveillance of sentinel cattle and vector midges during both the seasonal and interseasonal (overwintering) periods of BTV activity at a dairy farm; and
2. the combination of field and laboratory studies to investigate the potential role (if any) of transovarial transmission of BTV among *C. sonorensis*.

The overall goal of these studies was to further characterise the interseasonal maintenance and dynamics of BTV activity throughout the entire calendar year in a virus-endemic region, both in vector insects and sentinel cattle.

## Materials and methods

### Vector population ecology and infection studies

As previously described (Mayo *et al.* 2014a), a longitudinal study of BTV infection of *C. sonorensis* midges and sentinel cattle on a dairy farm in Northern California began in August 2012. Briefly, traps were placed at 0.25 km intervals along 4 transects, each directed at 90° quadrants from the outside perimeter of the farm's waste-water lagoon. For harvesting of vector midges, we operated CDC style traps without light and baited with dry-ice from dusk to dawn during both the seasonal (July-November) and interseasonal (December-June) periods and throughout the day only during the interseasonal period (Newhouse and Siverly 1966). Collections of *C. sonorensis* midges were made every other week for 52 weeks until August 2013, as previously described (Mayo *et al.* 2014a). Collection of adult midges was repeated during a portion of the inter-seasonal period of 2014 (January-March) on the transect, where 32 parous females had been collected in 3 traps during February 2013, and on another transect where no inter-seasonal activity of midges was detected during 2013. *Culicoides* midges were the only species identified in this trapping effort and were sorted by sex and parity and then pooled into groups of 20 midges from the same collection date and trap number (Wirth and Morris 1985). Cattle resident on the dairy farm were used as sentinels to detect circulation of BTV from August 2012 until August 2013, as previously described (Mayo *et al.* 2014a).

### Studies to elucidate potential for transovarial transmission of BTV in vectors

Field and laboratory studies were performed to further understand the potential role of transovarial (vertical) transmission of the virus in vectors in the interseasonal overwintering of BTV infection in temperate regions (Osborne *et al.* 2015). *Culicoides sonorensis* larvae were collected bi-weekly from a waste-water lagoon of a dairy farm in Northern California that was previously determined to have substantial seasonal BTV transmission (Mayo *et al.* 2014a). Larvae were identified under a dissecting microscope and categorized and counted by instar, according to head capsule size (Mullens 1987). In addition to field samples, a colony of *C. sonorensis* (VR strain, initially from a San Bernardino Co., CA dairy in 1995) was established and reared using methods previously described (Osborne *et al.* 2015). A strain of BTV serotype 17, previously isolated from a sheep in Modesto County (virus designated as 'Modesto 22'), was used for oral infection of female midges at 2-3 days of age. Engorged females were transferred to 1 of 4 holding containers in groups of < 20 midges. A moist filter paper pad was provided daily to the females for oviposition, beginning 2 days after the initial blood/virus feeding. Every 2 days, deceased females and an aliquot of approximately 10% of eggs were collected and stored in RNAlater® Solution (Ambion®, Grand Island, NY, USA) at 4°C for 24 hours prior to freezing at -80°C until assayed. The remaining eggs were transferred to larval rearing containers. Each egg group was allowed to mature through larval, pupal, and adult stages, and subsets (approximately 30%) of each stage were collected for virological assay. The presence of BTV RNA within samples collected from all studies was determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) amplification and detection of the BTV S10 gene, as previously described (Mayo *et al.* 2012).

## Results and conclusions

The main objective of these studies was to describe the inter-seasonal maintenance and seasonal/inter-seasonal dynamics of virus infection of vector midges and cattle resident on a dairy farm in Northern California with endemic but highly seasonal BTV infection. In recently published studies (Mayo *et al.* 2014, Mayo *et al.* 2014b, Osborne *et al.* 2015), we have, for the first time:

1. documented the presence of BTV in pools of parous female *C. sonorensis* midges collected during the inter-seasonal period (in both 2013 and 2014), which potentially explained the mechanism for overwintering of BTV on this farm; and
2. confirmed the lack of any evidence of vertical transmission of BTV to the progeny of virus-infected female *C. sonorensis* vectors, both in the laboratory and the field.

The detection of BTV in pools of parous female midges collected host-seeking during the day in the inter-seasonal periods of both 2012 and 2013 represents most likely the mechanism of virus-overwintering in long-lived female midges, which were infected during the prior seasonal period of virus transmission. It is further likely that these infected parous midges were less active in the fall and re-emerged in midwinter during a transient period of higher temperature (Mayo *et al.* 2014a).

The finding of BTV-infected parous midges during the overwintering period is globally relevant to temperate areas of the world and, potentially, to future incursive zones of BTV activity facilitated by climate change. Potential methods for the inter-seasonal maintenance of BTV on the high veldt of South Africa have been proposed by Nevill (Nevill 1971). These mechanisms were extensively discussed in a recent publication, and key points are reported in Table I (Mayo *et al.* 2014a).

In summary, our combined field and laboratory studies suggest that BTV is not readily transmitted vertically in *C. sonorensis*, and that persistence of

**Table I.** *Summary of proposed overwintering mechanisms and scientific evidence supporting or refuting mechanism for overwintering within temperate zones (Mayo* et al. *2014a)*

| Proposed overwintering mechanisms | Scientific evidence to date |
|---|---|
| Transovarial transmission of BTV in vector insects | This phenomenon has not been proven definitively for BTV with any *Culicoides* species in either field or laboratory studies. |
| | We found no evidence of vertical transmission of BTV to the progeny of *C. sonorensis* infected with BTV in the laboratory or in larvae, male or nulliparous female *C. sonorensis* collected in the field. |
| A complicated overwintering cycle involving an unidentified intermediate host | This mechanism is unlikely in California because *C. sonorensis* feed principally on ruminants. Blood meal analysis of *C.sonorensis* midges collected during these studies confirmed that these vectors fed only on cattle (*Bos taurus*) and Black-tailed deer (*Odocoileus hemionus columbianus*) (unpublished data). |
| Prolonged infection of ruminant livestock | This potential mechanism is highly unlikely as multiple recent studies confirm that viremia in BTV-infected livestock is transient, and the duration is considerably less than that of the approximately 6-month interseasonal (overwintering) period (reviewed MacLachlan and Mayo, 2013). |
| | Duration of the viremia in livestock that is infectious to vector midges is typically < 60 days. |
| Prolonged survival of adult *Culicoides* insects | This mechanism provides a plausible explanation for the overwintering of BTV in temperate regions, as female midges are infected for life but become infectious only after an extrinsic incubation period that is temperature dependent. |
| | Much remains to be learned regarding the temperatures that occur at potential refugia where adult midges may rest in nature during the interseasonal period, and their impact on midge survival. |
| An ongoing and slow/low-level cycle of infection between cattle and *Culicoides* midges | This mechanism also provides a potentially plausible explanation for the overwintering of BTV, however sentinel cattle at our study sites never became infected during the interseasonal overwintering period. |

the virus in long-lived parous female midges is a more likely mechanism for overwintering of BTV in temperate regions (Mayo *et al.* 2014a, Osborne *et al.* 2015). This conclusion is further supported by findings of our field studies on dairy farms in Northern California, where BTV infection has been detected only in parous female midges, and never in males or nulliparous females, as it would be expected had vertical transmission of BTV occurred frequently in the vector (Mayo *et al.* 2012, Mayo *et al.* 2014a, Osborne *et al.* 2015).

However, additional field studies are needed to confirm the precise mechanism of BTV overwintering in different temperate regions of the world. As the role and significance of other mechanisms of inter-seasonal maintenance of arboviruses can be influenced by virus strain, climatic influences, vector species, and strain.

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