

Vector Competence of British *Culicoides* spp. for BTV-8

Medreonet
3rd December 2009

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Introduction

Vector competence is important for assessing risk of Bluetongue introduction and likely spread patterns:

Requires screening of large populations as infection rates tend to be low

2008 preliminary work, addressed difficulties in carrying out *Culicoides* vector competence studies in the laboratory:

- Transport method
- Storage method post-incubation
- Virus presence/quantification post-incubation

2009 field season – low level of replication

Future work

2008 field season - method

Preliminary trials

Culicoides caught in the field, transported to Pirbright

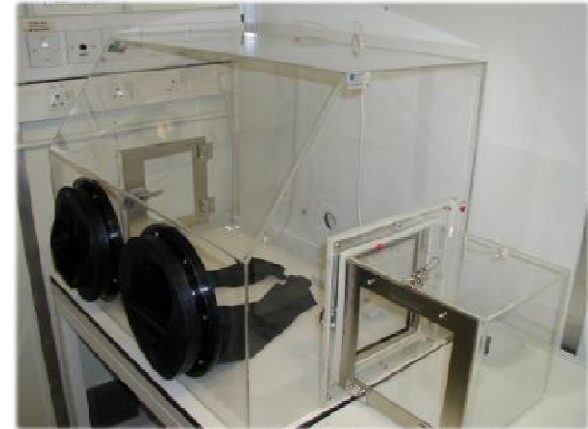
Pad-fed BTV-8 NL (E₁ BHK₂)/blood (1:1)

Sorted under CO₂ and incubated for 7 days

Stored in pools of ~20 midges

Homogenised using previously described method (Veronesi et al. 2008)

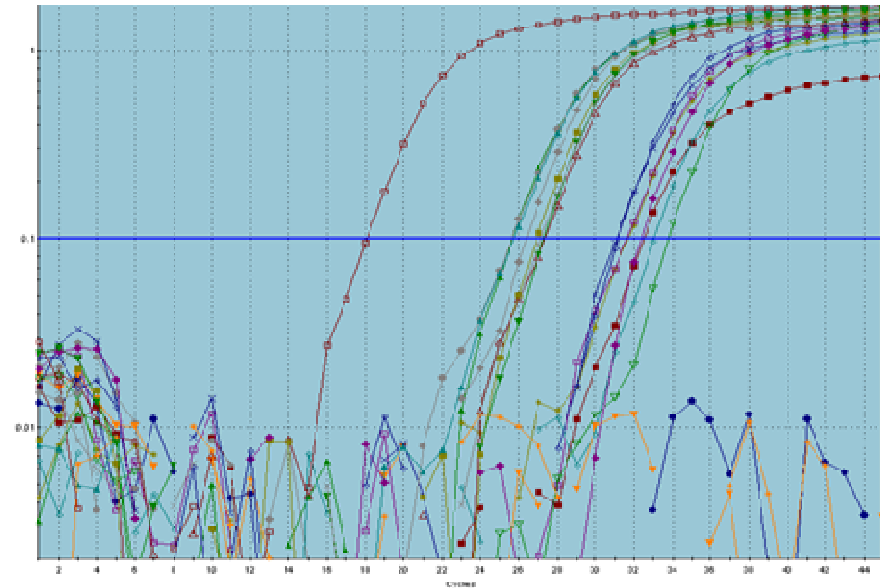
Isolation on BHK cells, confirmation by rRT-PCR



2008 field season – results

Processed and isolated:

- 153 x *C. impunctatus*
- 2 x *C. punctatus*
- 4 x *C. obsoletus* s.l.
- 212 x *C. imicola* (Corsica)

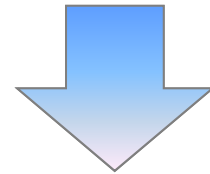


All negative via BHK-21 culture, so tested with rRT-PCR (Shaw assay)

- Provided high ct+ values (i.e. < amount virus)
- Inactivated virus / non-transmissible infections?
- ‘Transmissible’ *C. sonorensis* substantially lower ct+

Major obstacles

Wild caught midges



Transport (20-50% survival)

Midges exposed to virus



Feeding rate (20-50% feed using pleglet)

Midges taking a bloodmeal



Incubation (50-80% survival)

Stored midges



<10% midges competent

Competent Population

Transport method

Previously used cardboard pillboxes

Very high mortality

- Desiccation
- Rapid temperature shifts?



CIRAD method

- Polystyrene maintains temperature
- Maintains high humidity
- >90% survival for *C. imicola*
- 20-50% survival for *C. obsoletus* grp
(dependent upon postal system)



Cheaply and easily manufactured

2009 Storage method optimisation

Need to store infected midges during field season (quarantine restrictions)

Freezing at -70°C ? (e.g. Carpenter et al., 2006 - loss of infectivity approx 1 log TCID₅₀)

Storage at $+4^{\circ}\text{C}$ with antibiotics (in literature but species specific!)

Trialled antibiotic combinations of:

- 1) Penicillin
- 2) Streptomycin
- 3) Amphotericin B
- 4) Oxytetracycline

Most effective combination to date: 1+2+3 – no evidence of fungal growth up to 1 month post-storage.

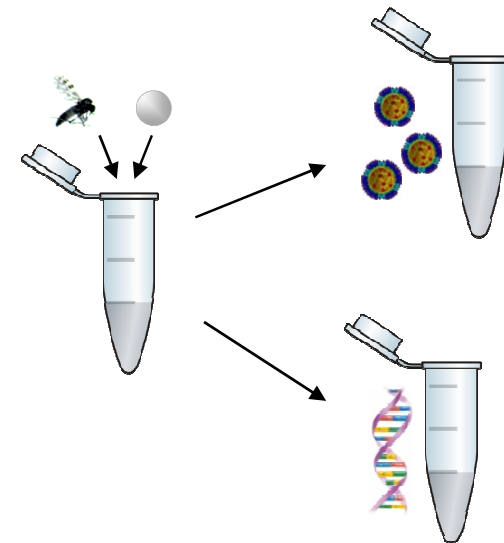
Virus determination methods

Stored midges individually in 96 well format (less contamination)

Homogenised in 96 well format with 3mm beads

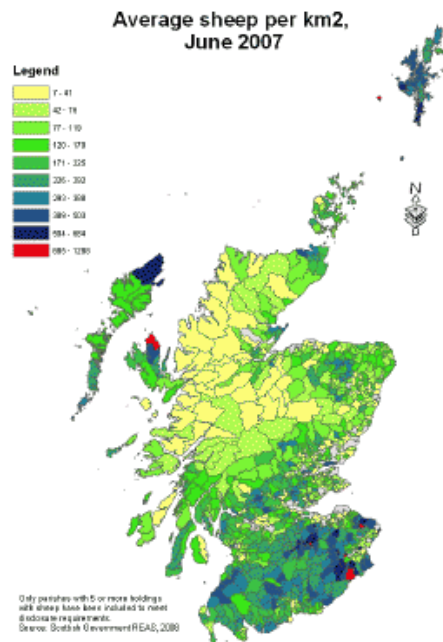
Supernatant removed for virus isolation (as for Veronesi et al. 2008)

Remaining pellet used for species specific multiplex PCR in 96 well format (Nolan et al., 2007 with chelex extraction) and 96 well gel



2009 field season

Red = Farm Collections
Blue = Sweep net



2009 field season

Site Code	Species			
	<i>C. impunctatus</i>	<i>C. obsoletus</i> grp	<i>C. pulicaris</i>	<i>C. punctatus</i>
TNLW	176	334	9	0
KKTN	29	55	1	4
BWBT	1	28	23	1
GLTN	30	0	0	0
MSTY	36	0	0	0
BOO	302	0	0	0
GLTL	5	42	5	0
CRTN	6	60	6	0
KNMR	6	36	6	0
APS	116	0	0	0
BKTS	0	486	0	0
Total	707	1041	50	5

2009 field season: Virus titrations (BHK-21)

Site Code	Species			
	<i>C. impunctatus</i>	<i>C. obsoletus</i> grp	<i>C. pulicaris</i>	<i>C. punctatus</i>
TNLW	176 (0)	334 (0)	9 (0)	0
KKTN	29	55	1	4
BWBT	1	28	23	1
GLTN	30	0	0	0
MSTY	36 (0)	0	0	0
BOO	302	0	0	0
GLTL	5	42	5	0
CRTN	6 (0)	60 (1+ : <i>C. scoticus</i>)	6 (0)	0
KNMR	6	36	6	0
APS	116	0	0	0
BKTS	0	486	0	0
Total	707	1041	50	5

Black = completed; Red = in storage

2009 field season: Virus titrations (BHK-21)

Rates of replication substantially lower than with Kosovo BTV-9 used by Carpenter *et al.*, 2006 despite very similar titre.

This is seen across *C. impunctatus*, *C. obsoletus* grp, *C. pulicaris* and *C. imicola* (from Corsica) but BTV-8 does replicate consistently in colony *C. sonorensis*.

Explanations:

- Population variation?
- Temperature during larval development?
- Other means of transmission (contact/mechanical)?
- Methodologies used (e.g. no *C. chiopterus*?).

Future work

Completion of processing (BHK-21 isolation/RT-PCR/KC isolation)

Assessment of RT-PCR vs cell based isolation in KC cells

Examining methods to demonstrate full dissemination of BTV

Multi-country studies

Acknowledgements



Scottish Government



Alison Blackwell (APS)



Stuart Piertney (U of Aberdeen)



CIRAD



Vector-borne Disease Programme (IAH)



Gert Venter (OVI)