Oral Susceptibility of

BTV-1, -2, -4 and -8 Spanish Strains in South African *Culicoides* Population

Introducción

BTV: Broad genetic variability

point mutations

reorganisations

pathogenic capacity very variable

even within the same serotype

Aim of the study: Analyse the oral susceptibility of *C. imicola* against different serotypes of BTV present in Spain

Material and methods

Viruses:

- BTV-1, -2, -4 and -8 (Central Veterinary Laboratory; Madrid (Spain))
- Mix with fresh sheep blood (1:1)
- Final titre:

From 1.2×10^5 $log_{10}TCID_{50}$ (BTV-2)

to 2.55×10^6 $log_{10}TCID_{50}$ (BTV-1)

Insects:

Culicoides ⟹ ARC-OVI centre (RSA)

March to April 2009

pools of 100 individuals

kept in 250ml card foam cups

stored: 23.5ºC in darkness

50-70% R.H.

3-4 days

Feeding Technique:

- NO nutrients or water 24h. before the bloodmeal
- Fed for 40’ on defibrinated sheep blood containing 1 serotye
- Blood engorged females:
  - kept in darkness
  - 5% sucrose solution
  - 10 (dEIP)
- Midges which survived:
  - 1.5ml microfuge tubes
  - stored at -70ºC

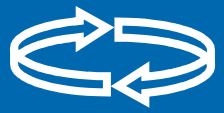
Processing of *Culicoides* and virological assays:

Midges assayed:

immediatly after blood feeding

after 10 dEIP

♀ homogenized in 100ml EMEM

15’ 

Supernatant ⟶ 1.5 ml microfuge tube 

100 ml EMEM+antibiotics+FBS

25 ml h.  100 ml BHK-21

three microtitre wells

microplates : 37ºC

5% CO_2

Observed for CPE 5–6 days

Virus Neutralization test:
serotype identity confirmed by microtiter virus-neutralization procedure using serotype-specific guinea pig's antisera

Virus Titration:
Original homogenates re-tested at serial 10-fold dilutions when CPE observed

Vector competence:

Titres ⟹ 2.5 $\log_{10}TCID_{50}$/midge ⟹ virus transmission

Other *Culicoides* species:

⇩ *numbers*

C. bolitinos
C. enderleini
C. nevilli

RESULTS

***Culicoides* survival rate:**

6063 midges fed

3573 (58.4%) survived 10 dEIP

Survival rate: 34-81% depending on the serotype fed

Virus recovery in midges tested immediately after feeding:

⇧⇧ contamination

Virus recovery and virus titre after 10 dEIP:

BTV-1
BTV-2
BTV-4
} recovered from *C. imicola*

BTV-8 not recovered

Highest recovery for BTV-2 (0.5%)

Titres of 2.5 $\log_{10}$TCID$_{50}$/midge demonstrated in BTV1 and BTV2

Other *Culicoides* species:

C. bolitinos
C. enderleini
C. nevilli

None of them tested positive to any serotype

	BTV-1	BTV-2	BTV-4	BTV-8
Virus titre of bloodmeal ($log_{10}TCID_{50}$/ml)	6.4	5.08	5.78	6.08
Species	+/tested	+/tested	+/tested	+/tested
C. Imicola Inf. Rate	1/490 (0.2%)	4/889 (0.5%)	1/404 (0.2%)	0/492 (0.0%)
Avg. virus titre/midge ($log_{10}TCID_{50}$/midge)	3.9	2.5 (1.2-3.2)	2.4	0.0
C. bolitinos	0/2	0/2	0/11	0/5
C. enderleini	0/1		0/1	0/8
C. nevilli				0/2

Virus recovery rates and titres in field colected *Culicoides imicola, C. bolitinos, C. enderleini* and *C. nevilli* manteined for 10 days at 23.5ºC after feeding on blood containig different serotypes of BTV

Discussion

- ✓ Virus recovered same serotype as the one they were fed

  ⇩ Infection rate not influenced by field infections

- ✓ *C. imicola* capable to become infected and transmit at least two of the four serotypes (BTV-1 and BTV-2)

  ⇧ 2.5 $\log_{10}$TCID$_{50}$/midge

- ✓ 2.4 $\log_{10}$ TCID$_{50}$ observed for BTV-4 should be more than enough to infect the salivary glands of the midge and act as a competent vector

- ✓ NO virus recovery could be obtained for BTV-8

  Refractive to infection ??? ⟶ Infection rate ⇩ 0.2%

- ✓ NO significant differences were found for any of the four BTV serotypes assayed

✓ NO virus isolation obtained from : *C. bolitinos*

C. enderleini

C. nevilli

number of individuals too low

<u>The current study shows low infection capacity for *C. imicola*</u>

Nevertheless….

…abundance, biting rate, survival after feeding, host preferences……. compensate the low infection rate observed and together with *C. bolitinos* are the **most competent vectors for *Orbivirus* in South Africa**

Considerations

- Field populations of *Culicoides* species can vary broadly in the genetic susceptibility to BTV infection
- Some populations of the same species might be completely refractory to infection by some serotypes of the virus

  So….

  …If we want to obtain epidemiological maps and predict futures outbreaks, oral susceptibility tests should be done at risk areas to asses *Culicoides* local population to the different strains of BTV serotypes

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