









"The emergence, persistence and evolution of bluetongue viruses in Europe since 1998"

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Bluetongue virus: History pre 1998

- First described in 18th century: confined to Africa
- > 1924 Cyprus, 70% mortality
- > 1943-47 Palestine and Turkey
- > 1948 USA
- > 1951 Israel and Asia
- > 1956 BTV-10 Eastern Mediterranean (Morocco, Spain, Portugal) 75% mortality in sheep, 17,900 killed in 4 months.
- > 1969 BTV-4 Cyprus
- > 1979-1980 BTV-4, Greece.
- European outbreaks were infrequent, short lived and involved only a single BTV serotype on each occasion.

BTV History: after 1998



- > 1998 multiple annual incursions started into Southern Europe.
- > 1999 -
 - > Multiple exotic serotypes arriving in Southern USA
 - New serotypes (BTV-2, BTV-7 in Australia)
 - Western vaccine-strains (BTV-2, BTV-10) in India (disease in native breeds – ~25% sheep fatality rates)
- 2006 BTV-8 in northern Europe (~25% sheep mortality)
- > 2010 Novel serotypes detection of BTV-25, 26, 27, 28....
- > 2014 BTV-4 arrives and spreads in eastern Europe.
- > 2015 BTV-8 reappears in central France (but low virulence)

Culicoides vector species from Europe, Africa and the USA





C. impunctatus (pulicaris complex)





Bluetongue: clinical signs



Haemorrhages in the dermis of a BTV-8 infected sheep, at 8 d.p.i (peak of viraemia)



Science

The first recorded outbreak of Bluetongue in Northern-Europe started in the Netherlands during 2006 – efore spreading across the rest of Europe outh ulcers

G BOSQUET SNGTV

Coronitis

Conjunctivitis

4-week old calf with behavioural & locomotory problems.

Brain with cerebral hemispheres replaced by a fluid filled sac. (Wouda et al 2008)



Bluetongue: Virus structure

Organisation of the BTV capsid and genomic dsRNA



The outer surface of the BTV virus particle VP and VP5 - Encoded by segments 2 and 6 (Cryo-EM and x-ray crystallography reconstruction)

> 120 VP5 trimers, encoded by Seg-6

60, VP2

trimers,

Seg-2

encoded by

erinary Medicine and Science

6



The emergence of Bluetongue In Europe Since 1998

Why is this happening now?

Introductions of different BTV strains into Europe and North Africa 1998 to end of 2007





2002200620002002 2006





The effect of temperature on incubation in the insect : extrinsic incubation period





BTV -1 polymerase activity



Temperature



Recent climate warming and Bluetongue outbreaks in Europe





The recent emergence / detection of novel BTV serotypes

Comparison of Seg-2 sequences for 27 BTV types









BTV Segment 3 phylogenetics







- BTV-26 identified in 2010 in sheep from Kuwait
 - caused only very mild disease in sheep.
 - replicates and persists in goats but no clinical signs.
 - detected in urine.
 - contact transmission was detected between goats, in the absence in insects.
 - BUT did not infect adult Culicoides or Culicoides cell line (KC cells).

BTV-26 transmission study in goats:





3 infected goats 3 in contact controls 4 barrier control goats 1ml Subcutaneous inoculation BTV-26 KUW2010/09 BHK2 10⁶ TCID₅₀/ml ____



Samples of serum, EDTA blood and nasal/ocular swabs were taken throughout the study



Generation of BTV-1/BTV-26

mono-reassortants by reverse genetics

Virus	Seg-1	Seg-2	Seg-3	Seg-4	Seg-5	Seg-6	Seg-7	Seg-8	Seg-9	Seg-10
rBTV-1 _{26 S1}										
rBTV-1 _{26 S2,6,7}										
rBTV-1 _{26 S3}										
rBTV-1 _{26 54}										
rBTV-1 _{26 S5}										
rBTV-1 _{26 56}										
rBTV-1 _{26 57}										
rBTV-1 _{26 S8}										
rBTV-1 _{26 S9}										
rBTV-1 _{26 S10}										

Panel of generated BTV-1/BTV-26 mono-reassortants



Pullinger et al., (2016). In PLoS ONE. doi: 10.1371/journal.pone.0149709

Replication of BTV-1 and BTV-26 reassortants in BSR cells





MOI = 0.04, Values are means \pm SEM of 3 experiments, with 2 determinations each

Replication of BTV-1 and BTV-26 reassortants in KC cells





BTV-26 proteins restricting replication in *Culicoides* cell cultures (KC cells)





Binding of purified BTV virus particle to BSR and KC cells





Binding of purified BTV-1 or BTV-26 virus particles to BSR or KC cells after 1hour at 4°C.



Summary concerning the BTV outbreaks in Northern Europe



- BTV-8 (same lineage) re-emerged in France in 2015 and the outbreak still appears to be spreading. How did it persist through 5 'free' years ?
- Why so few clinical cases, this time? Has the virus or host population changed?
- BTV-4 also spread north from southern Greece , as far as Austria, Slovakia, Romania and Hungary.
- The 'at risk'/endemic zone for BTV may therefore be spread further north from the Mediterranean region.
- The increased proximity may represent an elevated risk of reintroduction to northern Europe.
- > Unlike the 2007 outbreak vaccine is available to farmers.
- > The risk of BTV incursion into UK population is currently assessed as **LOW**.
- It is likely that new BTV strains (and other arboviruses) will continue to spread into Europe.

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