Implications of Genetically Novel Bluetongue Viruses

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Outline

• History
  – Global epidemiology and episystems
  – Recent events including expansion/alterations in global range; Europe and North America
    – Improved diagnostics and understanding

• Novel BTVs
  – Genetic diversification of field strains of BTV
    – Distinction of epidemic versus endemic infection
  – Identification of new serotypes (25 – 27)

• Implications
  – Alternate episystems/transmission/viruses
    – Potential impact on surveillance
  – False negative RT_PCR test results
The Cycle of Bluetongue Virus Infection: a Non-contagious Infection

- *Culicoides* sp. = biological vectors but few of > 1000 global species = vectors of BTV

- All ruminants plus dogs and African/Eurasian carnivores

- Prototype *Orbivirus*, family *Reoviridae*
  - Segmented genome ds RNA
  - 24 “traditional” serotypes; 3 additional “new” ones since 2008
The Global Epidemiology of BTV Infection

• Exists throughout tropical and temperate regions of the world
  – Disease occurs at the upper and lower limits of the global range of BTV, in “incursional” areas

• BTV exists in relatively distinct global ecosystems of different *Culicoides* vectors with specific virus strains/serotypes
Global Distribution of BTV

Serotype diversity of BTV
Global Distribution circa 1990

Gibbs and Greiner, 1994
Different species of *Culicoides* vector disseminate different serotypes of BTV in distinct global ecosystems

Courtesy of U. Balasuriya; Maclachlan and Osburn, *JAVMA*, 2006
Bluetongue: Ecosystem Change?

- **North America**
  - Serotypes 10, 11, 13, 17 in much of US; BTV- 2 in south east but now CA; since 1998, 10 new BTV serotypes in Gulf Coast region
    - BTV 1,3,5,6,9,12,14,19,22,24; likely translocation from adjacent Caribbean ecosystem
- **Australia and Middle East**
  - Appearance of novel serotypes
- **Europe**
  - Emergence of virulent bluetongue in both Mediterranean and northern Europe since 1998 and 2006 respectively
    - Palearctic *Culicoides spp.* as vectors - *C. obsoletus* etc
Bluetongue in Europe: 1998 -

- Mediterranean Basin, 1998
  - Wind-borne insects via North Africa & Middle East, then spread by resident midges

- Northern Europe 2006
  - BTV-8 virulent – origin?
  - BTV-6, 11, 14 = South African vaccines
  - Virus transmission by Palearctic species of *Culicoides* midges
BTV 1, 2, 4, 9, 16 in the Mediterranean Basin – BTV-8 spread widely BUT Northern Europe free as of 2012 (BTV-14 still in Eastern Europe)
Genetic Diversification of Field Strains of BTV

• Distinguish “virgin-soil” epidemic like BTV-8 in Europe to long-term endemic regions like California

• Mechanisms
  – Genetic drift and founder effect
  – Genetic shift and gene reassortment

Balasuriya et al., *Vet Microbiol* 2008
GENE REASSORTMENT INDEPENDENT OF SEROTYPE; FIELD STRAINS OF BTV FROM CALIFORNIA

DeMattos et al., *J. Virol* 1996; Pierce et al., *Virus Res* 1998
Serological and Virological Detection of Field Strains of BTV

• Serological = cELISA

• Virological = group-specific RT-qPCR now standard
  – 2014 OIE Manual incorporates the NS3 gene-based assay (Orru et al., 2006; Hoffman et al., 2008)
  – Virus isolation still included in Manual

• Surveillance – Manual prescribes sentinel cattle
BTV Infection can be Prolonged in Ruminants; Association of Virus with Erythrocytes

Viral RNA in blood

Serum antibodies

Viremia

5-7 months

3-5 months

Positive result

2 4 6 8 10 Days after infection

2 4 6 8 Weeks

4 6 8 Months

Courtesy of M. Eschbaumer and B. Hoffmann

Adapted from Barratt-Boyes and Maclachlan, *J Am Vet Med Assoc* 1995
Figure 1. Duration of viremia and virus detection in semen of mature bulls infected with laboratory adapted Bluetongue virus serotype 1 (BTV-1). qRT-PCR = quantitative reverse transcription polymerase chain reaction.
Table 4. Comparison of the performance of quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays (including commercial kits) for the detection of Bluetongue virus (BTV) in semen from experimentally infected bulls.

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<th>S1</th>
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<th>K1</th>
<th>K2</th>
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<tr>
<td>Negative</td>
<td>66.2% (45*/68)</td>
<td>66.2% (47/68)</td>
<td>64.7% (44/68)</td>
<td>66.2% (10/68)</td>
<td>67.6% (46/68)</td>
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<tr>
<td>Positive</td>
<td>33.8% (23†/68)</td>
<td>30.9% (21/68)</td>
<td>35.3% (24/68)</td>
<td>33.8% (23/68)‡</td>
<td>32.4% (22/68)</td>
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* Denotes the number of samples that were tested in this assay that gave negative results.
† Number of samples giving positive results.
‡ Does not include 35 samples that gave false-positive results.
NEW SEROTYPES OF BTV
BTV SEROTYPE 25
“Toggenberg Orbivirus”

- Identified in healthy Swiss goats by RT-qPCR (Hoffman et al., 2008)
  - Not grown in embryonated eggs, cell culture, mouse brain
- Subclinical, persistent infection (> 25 months) of goats in Germany, Switzerland, and northern Italy
  - Circulating since 1998; high herd seroprevalence (100%)
  - Goats preferred host, sheep less susceptible, not wildlife?
  - High alpine pasturing a risk factor but transmission route?
SEROTYPE 26

- Novel BTV identified in sheep/goat flock in Kuwait in 2010
- Mild disease in sheep and low RNA levels, no disease in goats but high RNA (like BTV-25)
  - BTV-26 propagated in cell culture, not Culicoides midges
- Contact transmission, natural epidemiology?
BTV SEROTYPE 27

Detected by RT-PCR in healthy goats in Corsica in 2014 – high infection rate in goats, not cattle or sheep

Propagated in cell culture with difficulty, most related to BTV-25

No disease in experimentally inoculated goats, sheep or cattle (S. Zientara, pers. comm.)
POTENTIAL IMPACTS OF THE DISCOVERY OF NOVEL BTVs ON INTERNATIONAL REPORTING OF BT

• Animal surveillance
  – Small ruminants not cattle (sentinel cattle prescribed by OIE)
  – No disease so clinical surveillance not useful
  – Different epidemiology = persistent infections, horizontal transmission and lack of requirement for vector

• Entomological surveillance
  – What if these viruses do not require Culicoides midges?

• Laboratory Surveillance
  – Infected animals seroconvert by cELISA (weakly)
  – Difficult or not possible to isolate new viruses
  – False negatives with several widely used RT-PCR assays