Schmallenberg virus: diagnostic tools

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AHVLA:
An executive agency of the Department of Environment, Food and Rural Affairs (DEFRA), UK

A group of 16 laboratories (plus offices) all over the UK.

AHVLA activities include:
- National (and international) reference labs
- Emergency disease response (notifiable diseases)
- Horizon scanning for emerging diseases
- Endemic disease services
- Research and development
  - Test development/improvement
  - Applied research
Bunyaviridae

- ssRNA segmented genome
- < 300 distinct viruses in 5 genera
  - Orthobunyavirus
    - Simbu group
      - Akabane virus
      - Shamonda virus
    - Bunyamara group
    - California group
  - Hantavirus
    - Hantaan virus
  - Nairovirus
    - CCHF
  - Phlebovirus
    - Rift valley fever
  - Tospovirus (plants)
Schmallenberg virus deep sequencing at FLI, Germany

Hoffmann et al., EID, in press 2012
The UK virus is highly similar to the one detected in continental Europe
A similar result was obtained for the L-gene

Thus, Orthobunyaviruses are a very heterogeneous group with many members belonging to the Simbuviruses. Individual viruses, however, are fairly stable
Samples tested (for what purpose)

• Acutely “diseased” (infected) animals
  – Blood for virus detection
  – *Short duration of viremia; no persistence of virus (to current knowledge)*

• Post-mortem to investigate malformations
  – Brain tissue for virus detection
    • *Detection problem (hit & run) vs immunity*
  – Antibodies in fetal fluid

• **Screening animals for previous infection**
  – Serum for antibodies
  – *can be used to delineate immunity*

• **Analysis of tissues**
  – For presence of virus
  – *Necessary for SBV?*
Validation (modified from OIE)

Validation is the evaluation of a process to determine its fitness for a particular purpose. An assay validated for an infectious disease yields test results that identify the presence of a particular analyte (e.g. components of an infectious agent or antibody induced by it) and allows predictions to be made about the status of the test subjects. The term “validated assay” elicits various interpretations among laboratory diagnosticians [laboratory researchers] and veterinary clinicians.

Variables can be grouped into three categories:
(a) The sample – host/organism interactions affecting the composition and concentration in the sample;
(b) the assay system – physical, chemical, biological and human factors affecting the capacity of the assay
(c) the test result – the capacity of a test result, derived from the assay system, to predict accurately the status of the individual or population relative to the analyte in question.
QC systems (examples)

• **ISO 9001**
  – Quality management system to demonstrate the ability of an organization to consistently provide products that meet customer and regulatory requirements

• **ISO 17025**
  – Competence of testing and calibration laboratories
  – Using standard methods, non-standard methods and laboratory developed methods
  – **Validation of methods**
    • Validation is the confirmation …… that the particular requirements for a specific intended use are fulfilled
    • The validation shall be as extensive as is necessary to meet the needs of the given application
Virus detection assays

- **Virus isolation** – *(gold standard in virus diagnostic?)*
  Generally considered necessary to establish the link between a new virus and a disease
  Very laborious – and often impossible
  Not suitable as a diagnostic tool for SBV

- **Ag detection** –
  Detection of virus
  Limited sensitivity despite the enzyme enhancements
  Only as a post-mortem IHC tool

- **Nucleic acid detection** – *the next gold standard*
  Direct evidence, rapid, sensitive, flexible
  • RT-PCR
  • qPCR (one step/tube)

- **Ab detection** – *only an indirect trace of previous infection*
  • ELISA: rapid, cheap, sensitive
  • NT: more laborious, highly specific
Virus isolation (KC, BHK, Vero)
Ag detection, Schmallenberg virus, IHC GD/CVI-Lelystad

Lamb brain tissue (cerebellum) SBV Immunostaining (using a simbu serogroup Mab)
Toolbox molecular detection

• qPCR:
  – first generation FLI PCR – L segment
  – second generation FLI PCR – S segment
  – In house PCRs (several) – S segment
  – commercial PCR (LSI) – S segment
  – More to come ....

• Detection limit of 2nd generation qPCRs ≤50 copies

• RT-PCR (and sequencing) for confirmation only
Toolbox serology

- IFAT / IPX detection
  - Difficult to standardise or quantify

- ELISA systems (IDVet, in house)
  - First generation built on virus;
    2\textsuperscript{nd} generation possibly on rec protein
  - Validation currently remains an issue
  - To be resolved in the near future
  - Certainly to test a herd/group status
Virus Neutralisation Test (*SNT/PRNT possible*)
(e.g. CVI-Lelystad)

- SBV isolate lamb brain tissue
- Vero cells (for cpe)
- Culture
- Cell staining
- Applied for cattle and sheep sera
- Applicable to all species
- Specificity, 99.4%, tested with archived serum samples
- Sensitivity >92%, based on notified farm field samples
- Validation relatively straightforward
Summary SBV tests

- A comprehensive set of tests that is suitable to cover all aspects of infection, virus detection and control of disease has been set up across Europe
- These tests are both sufficiently sensitive and specific to fulfill their objectives
- Particularly the qPCR-based molecular detection assays the virus and the VNT are suitable to identify the virus or infection of individual animals
- In a rapidly changing environment more tests are likely to enter the market soon, which will complement the toolbox further.
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