Schmallenberg virus: diagnostic tools

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Animal Health and
Veterinary Laboratories
Agency

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

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Bunyaviridae

- - ssRNA segmented genome
- < 300 distinct viruses in 5 genera
  - Orthobunyavirus
    - Simbu group
      - Akabane virus
      - Shamonda virus
    - Bunyamera group
    - California group
  - Hantavirus
    - Hantaan virus
  - Nairovirus
    - CCHF
  - Phlebovirus
    - Rift valley fever
  - Tospovirus (plants)

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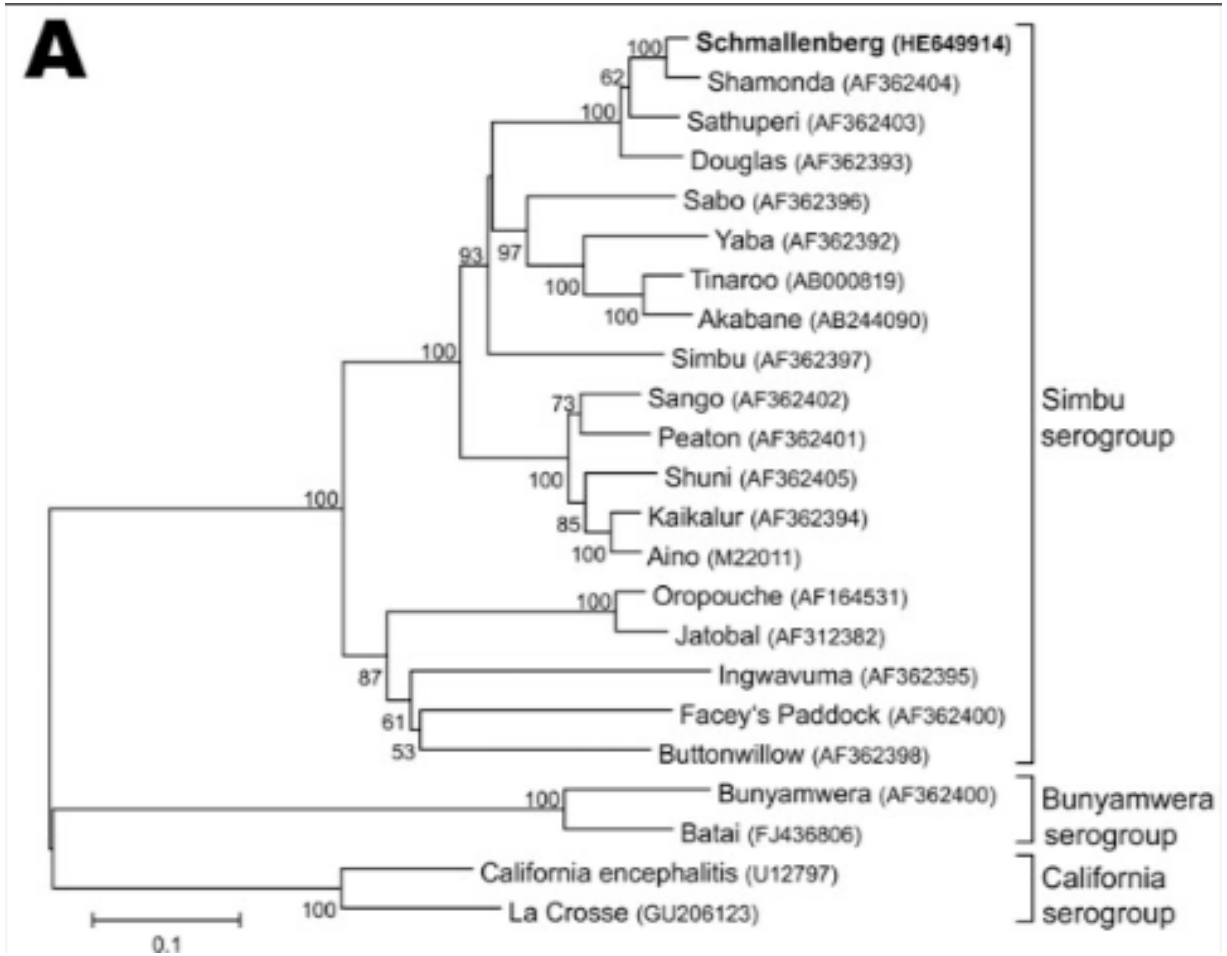
Schmallenberg virus deep sequencing at FLI, Germany



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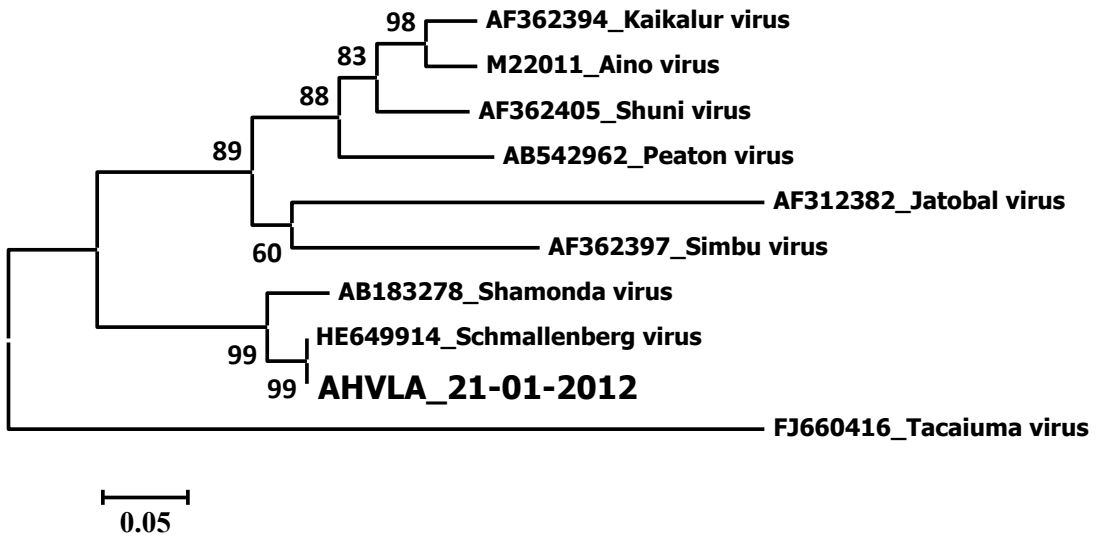
Hoffmann et al., EID, in press 2012

Phylogenetic tree constructed using a partial sequence of the N gene



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

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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 ?*

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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.

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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

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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

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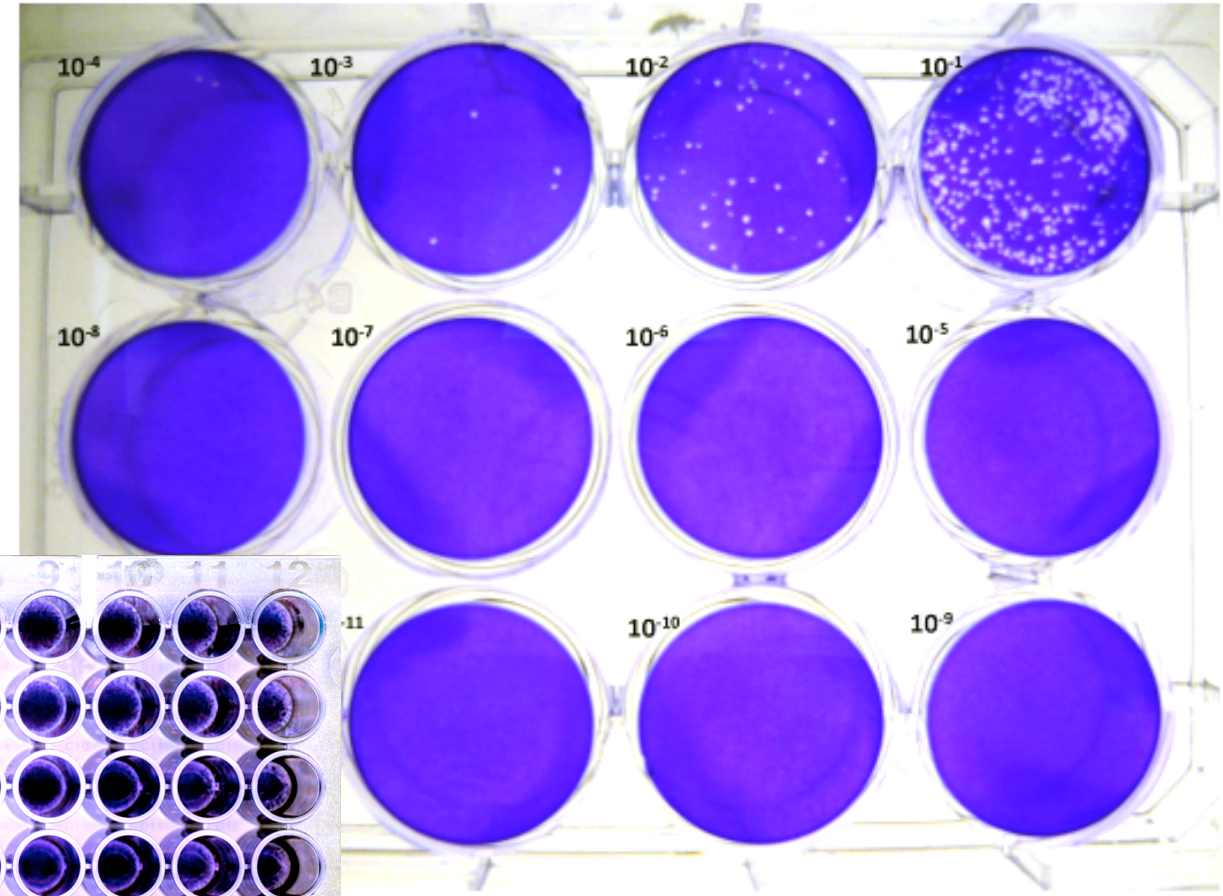
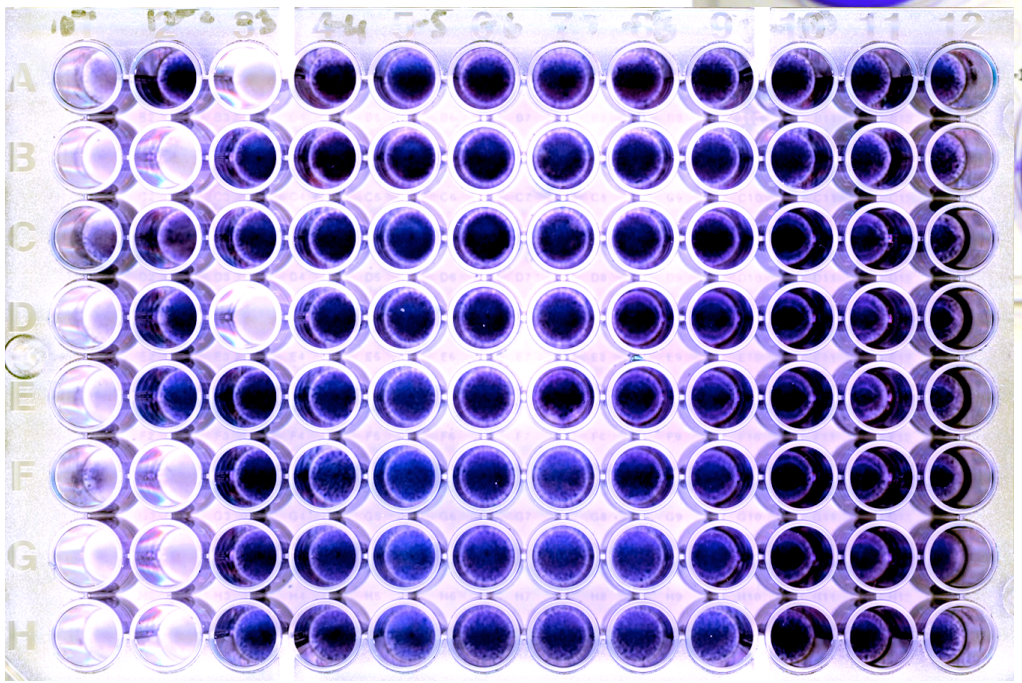
Virus isolation (KC, BHK, Vero)

Vero



BHK



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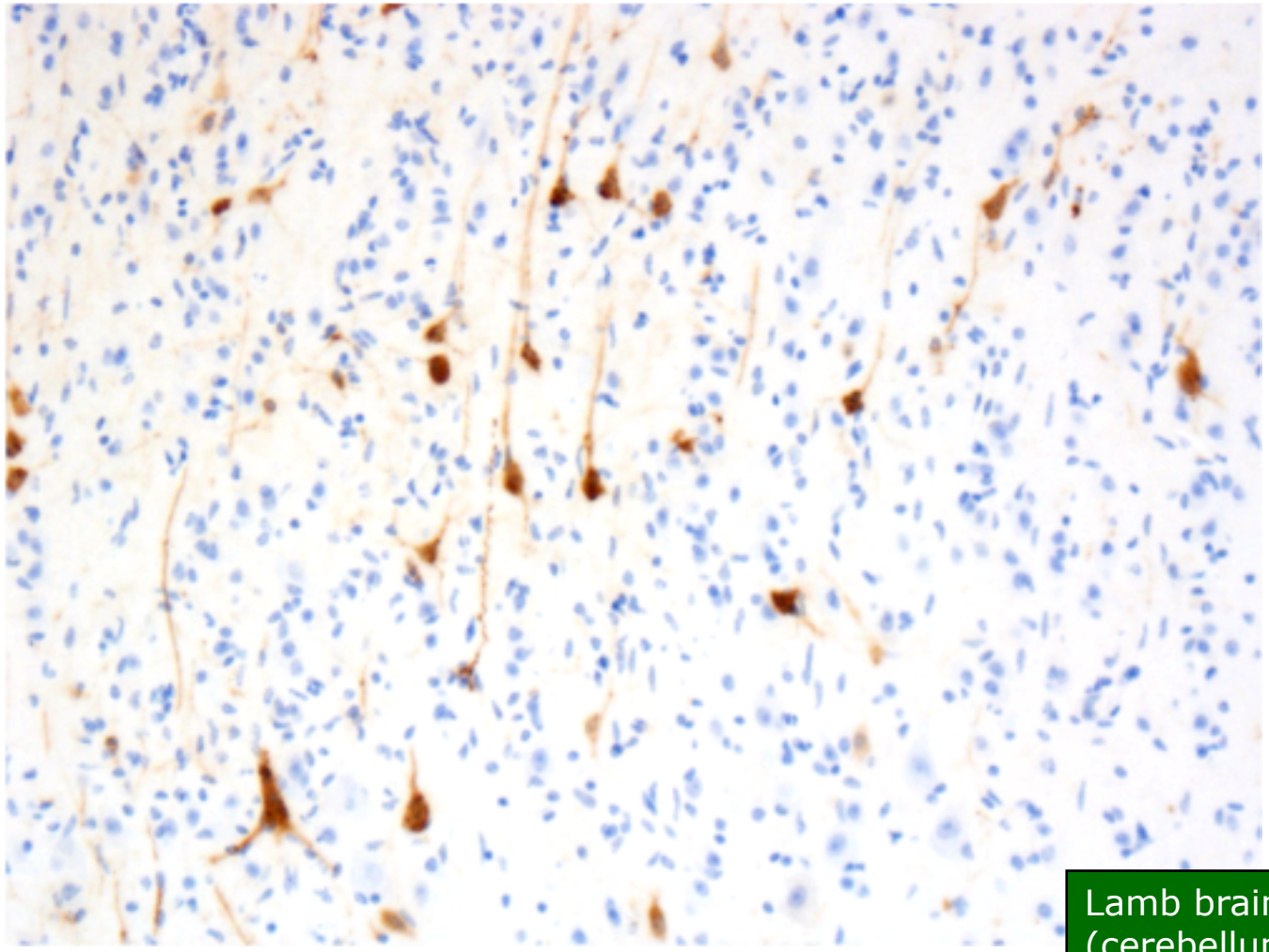
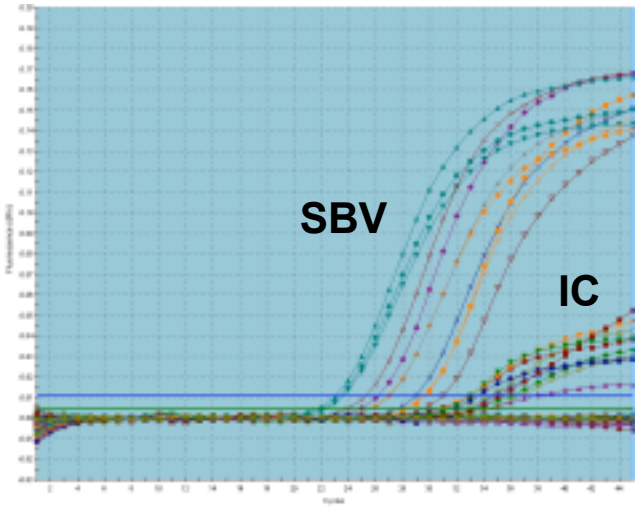
Ag detection, Schmallenberg virus, IHC GD/CVI-Lelystad



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

SBV N-gene

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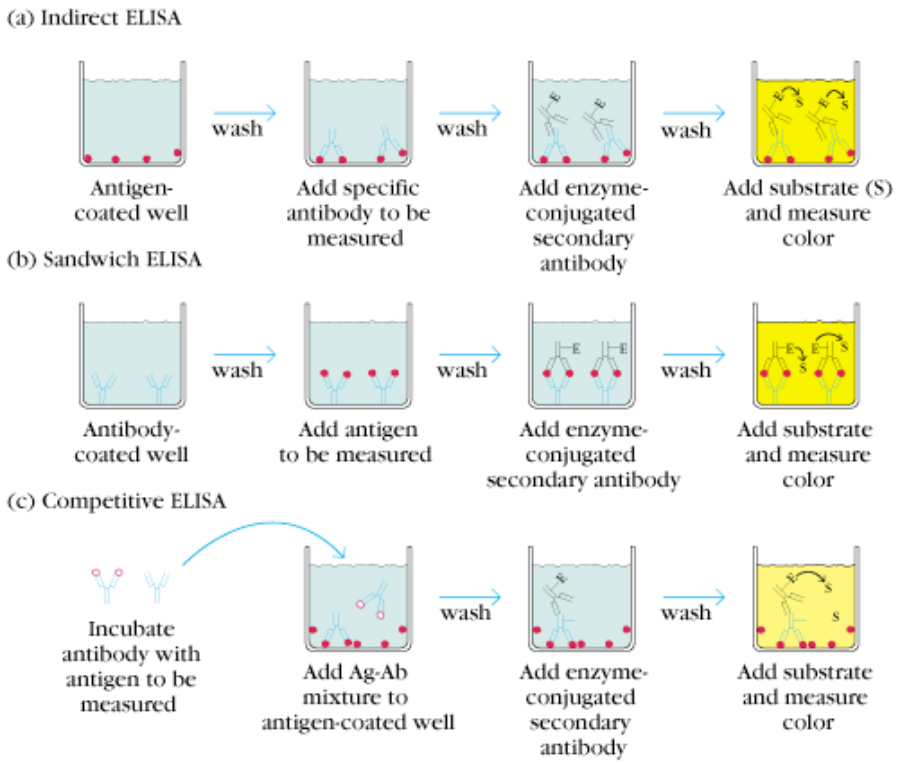
Toolbox serology

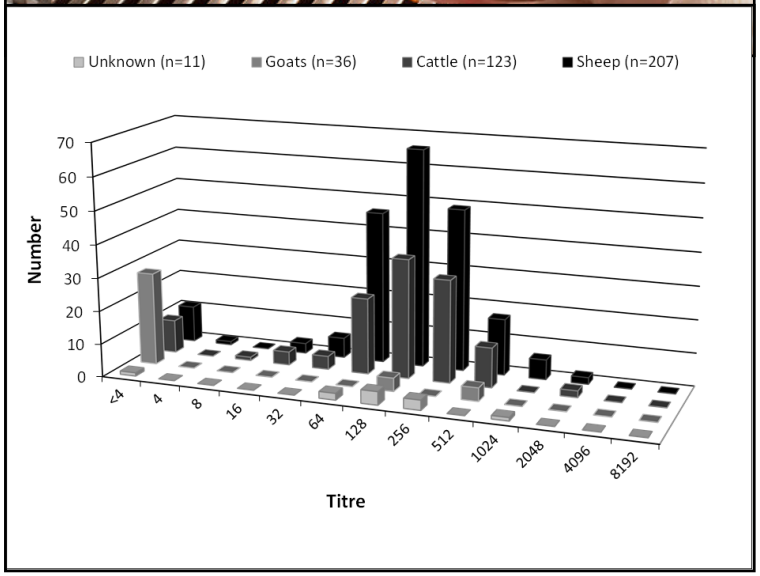
- **IFAT / IPX detection**
  - Difficult to standardise or quantify

- **ELISA systems (IDVet, in house)**
  - First generation built on virus;
    2nd 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 straight forward**



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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