Better Training for Safer Food BTSF

Programme Animal Health Prevention and Control of Emerging Animal Diseases

## Detection of emerging animal infectious diseases

etienne.thiry@ulg.ac.be

Agenda

- *OIE terrestrial manual*
- *Good practices in field sampling and specimen delivery*
  - **General considerations**
  - **Community reference laboratories (CRL/EU-RL)**
- *Diagnostic assays*
  - **Conventional direct and indirect tests**
  - **New generation tests (real time PCR, high throughput sequencing)**
  - **Case study: emerging Schmallenberg virus infection**

Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

- *« OIE Terrestrial manual »*

- http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/
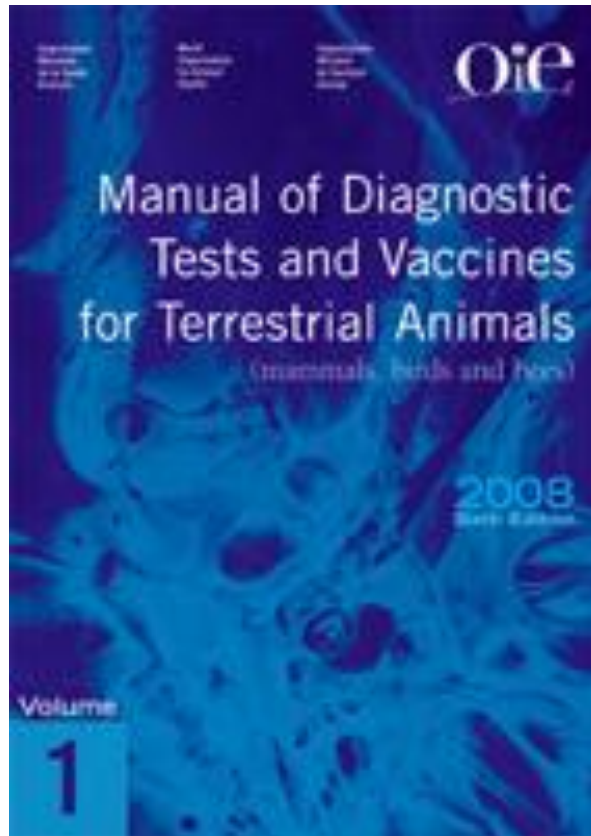
OIE « codes » and « manuals »

- **Terrestrial Animal Health Code**

- **Aquatic Animal Health Code**

- *standards for the improvement of animal health and welfare and veterinary public health worldwide, including through standards for safe international trade in terrestrial animals (mammals, birds and bees) and their products*

- **Manual of Diagnostic Tests and Vaccines for Terrestrial Animals**

- **Manual of Diagnostic Tests for Aquatic Animals**

- *provide internationally agreed diagnostic laboratory methods and requirements for the production and control of vaccines and other biological products*

GOOD PRACTICES IN FIELD SAMPLING AND SPECIMEN DELIVERY

General considerations

Community reference laboratories (CRL/EU-RL)

COLLECTION AND SHIPMENT OF DIAGNOSTIC SPECIMENS

- *General principles*
  - **Samples for disease diagnosis, disease surveillance, health certification or monitoring the response to treatment or vaccination**
  - **Samples be appropriate for the intended purpose, and adequate in number and amount to provide statistically valid results**
  - **Samples carefully packaged, labelled, and transmitted to the laboratory by the fastest practicable method, with the appropriate temperature control**

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.1.01_COLLECTION.pdf

COLLECTION AND SHIPMENT OF DIAGNOSTIC SPECIMENS

- *Investigation of a clinical condition*
  - **Specimens collected should be representative of the condition being investigated and the lesions observed**

- *Surveillance and monitoring*
  - **Statistical sampling methods ensuring:**
  - **Representativity (random sampling)**
  - **Precision (size of sample)**

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.1.01_COLLECTION.pdf

COLLECTION AND SHIPMENT OF DIAGNOSTIC SPECIMENS

- *Packaging and transport of samples*
  - **Information and case history**
  - **Approval of shipment**
    in accordance with the dangerous goods rules (IATA Dangerous Goods Regulations)
  - **Transportation of specimens**
    Fast (48h) in refrigerated conditions
  - **Packaging**
    See Dangerous Goods Regulations
    IATA list of pathogens UN 2814 or UN 2900; other : UN 3373

- *Preservation of sample for prolonged storage*
  - **Serum banks (-20°C)**
  - **Pathogen banks (-80°C and liquid nitrogen)**

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.1.01_COLLECTION.pdf

EU-reference laboratories (EU-RL; previously CRL)

- *Information on analysis methods*
- *Organisation of comparative tests (proficiency tests) with the national reference laboratories (NRL)*
- *Research into new analysis methods*
- *Organisation of trainings and advanced courses*
- *Providing scientific and technical assistance to the Commission*

EU-reference laboratories

- *Bee health*
- *Equine diseases other that African Horse Sickness*
- *African Horses sickness*
- *Bovine tuberculosis*
- *Crustacean diseases*
- *Avian Influenza*
- *Newcastle Disease*
- *Bluetongue*
- *Foot and Mouth Disease*
- *Swine Vesicular Disease*
- *African Swine Fever*
- *Rabies (serology)*
- *Rabies*
- *Bivalve molluscs diseases*
- *Zootechnics (bovine breeding)*
- *Brucellosis*
- *Fish diseases*

DIAGNOSTIC ASSAYS

Conventional direct and indirect tests

New generation tests (real time PCR, high throughput sequencing)

Case study: emerging Schmallenberg virus infection
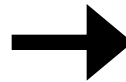
Nasal swabs

Diagnosis of viral infections

Direct diagnostic methods
(identification of virus, antigen, genomic sequence)

Indirect diagnostic methods
(identification of an immune response, antibodies)

Non specific
Observation of a virus infection, no identification

Cytopathic effect
Hemadsorption

Specific
Identification of a viral component

Detection of genome (biochips, sequencing)
Detection of proteins

Indirect Elisa
Indirect immunofluorescence
Seroneutralisation
Hemagglutination inhibition

Virus discovery – Metagenomics

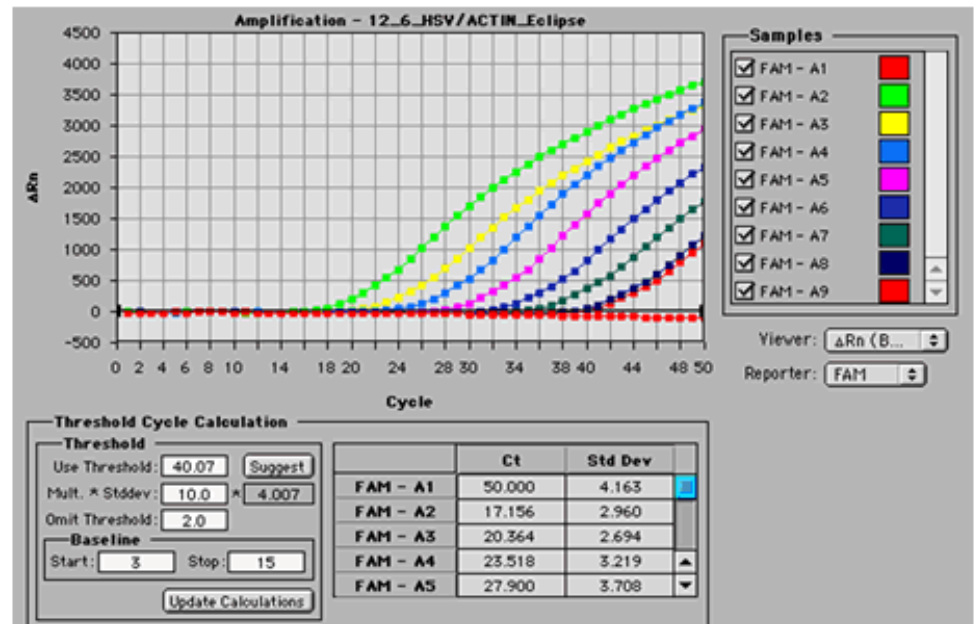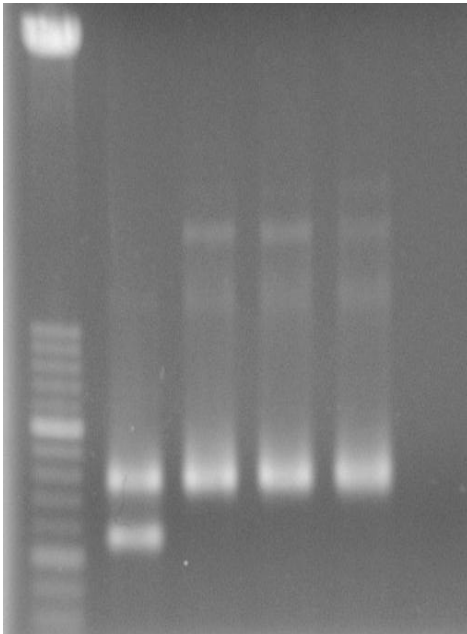# Non specific direct diagnostic methods

- *Cytopathic effect in cell culture*

- *Hemadsorption*
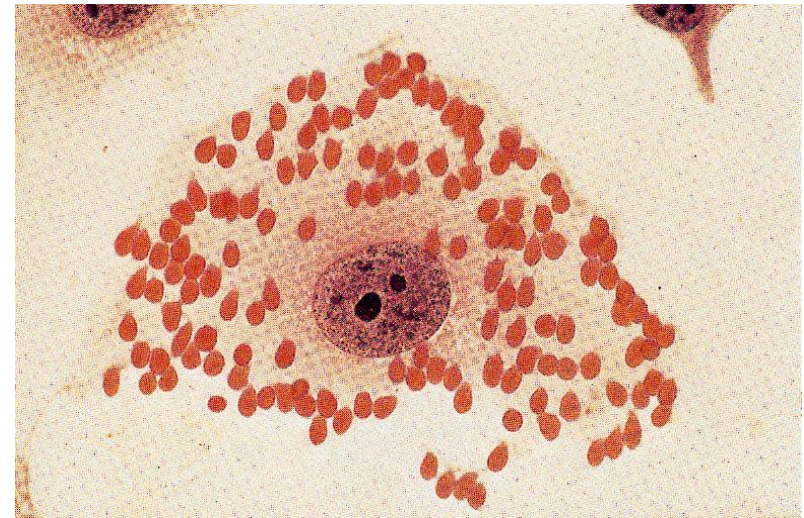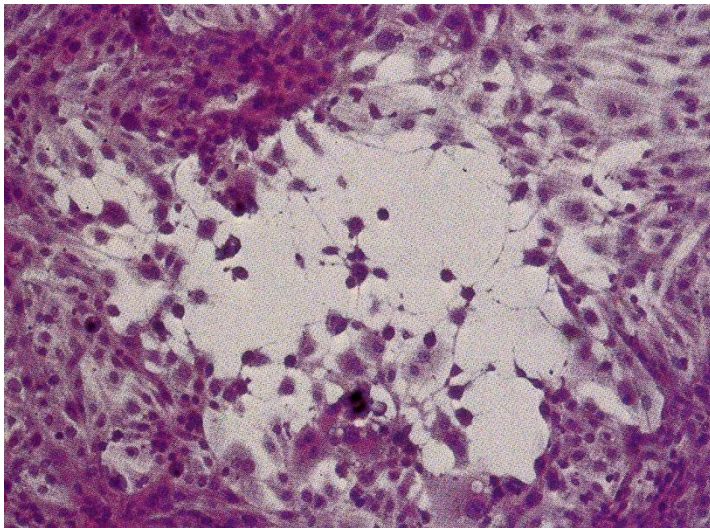
Specific direct diagnostic methods

- *(RT)-PCR*

- *Real-time PCR*

# Advantages and disadvantages of PCR methods

- *Conventional (RT-)PCR*
  - **Highly sensitive: detection of the genome, but not of the « infectious » agent**
  - **Specific : genotyping in one or two steps**

- *Real-Time PCR*
  - **Same characteristics**
  - **Quantification possible (equivalent genomes, extrapolated to viruses)**

New tools of viral diagnostics: biochips for the identification of multiple sequences
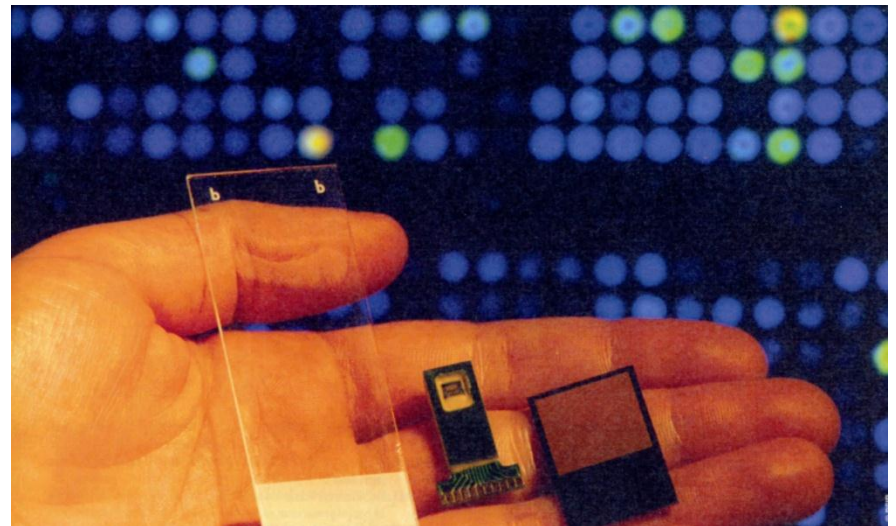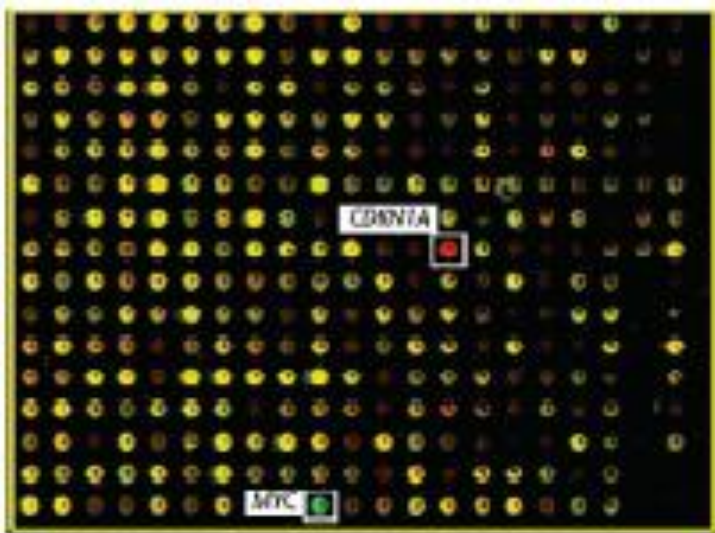
New tools of virus discovery: metagenomics with the use of high throughput sequencing
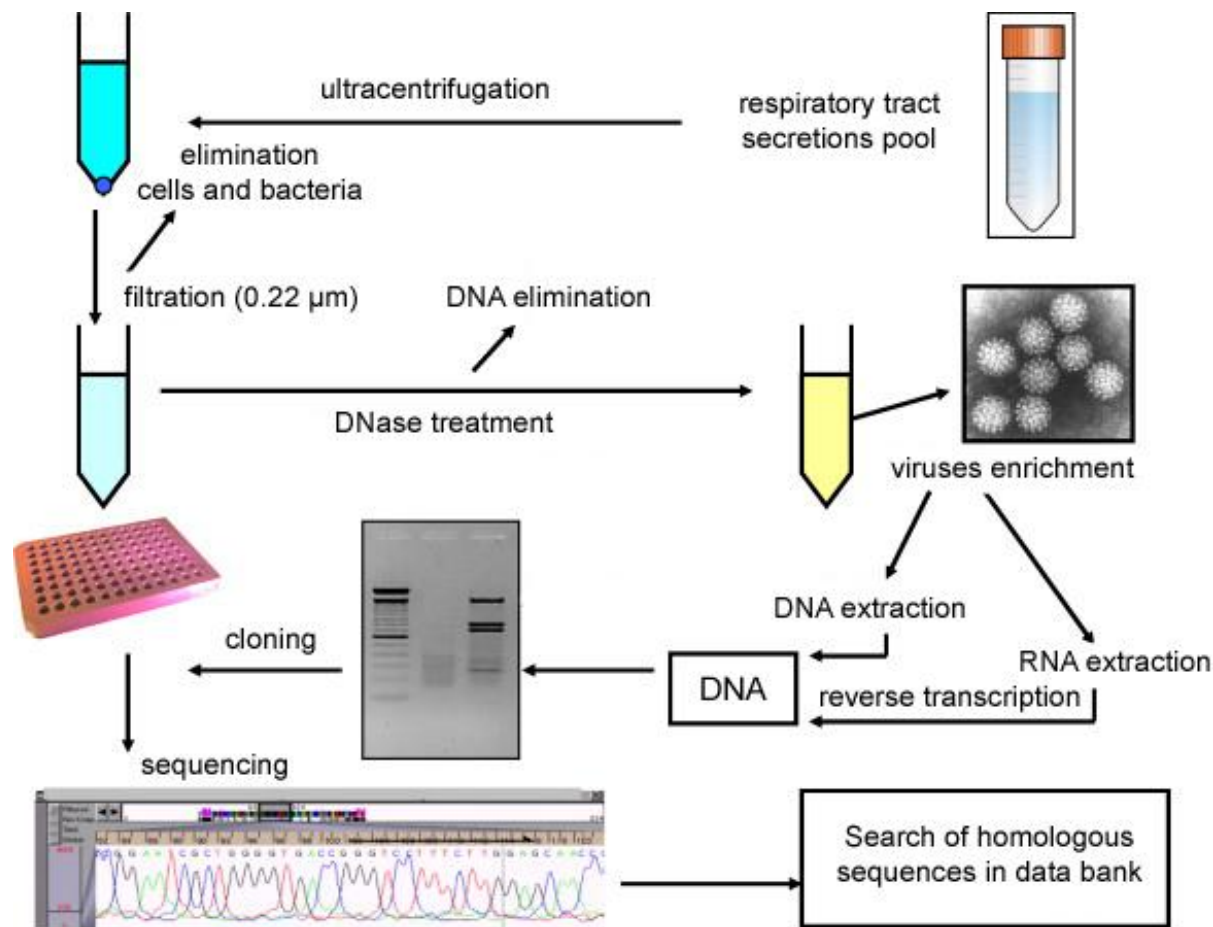
Emerging Schmallenberg virus infection: a case study

Novel Orthobunyavirus in Cattle, Europe, 2011

Bernd Hoffmann,[1] Matthias Scheuch,[1] Dirk Höper,
Ralf Jungblut, Mark Holsteg, Horst Schirrmeier,
Michael Eschbaumer, Katja V. Goller,
Kerstin Wernike, Melina Fischer,
Angele Breithaupt, Thomas C. Mettenleiter,
and Martin Beer

In 2011, an unidentified disease in cattle was reported in Germany and the Netherlands. Clinical signs included fever, decreased milk production, and diarrhea. Metagenomic analysis identified a novel orthobunyavirus, which subsequently was isolated from blood of affected animals. Surveillance was initiated to test malformed newborn animals in the affected region.

In summer and autumn 2011, farmers and veterinarians in North Rhine-Westphalia, Germany, and in the Netherlands reported to the animal health services, local diagnostic laboratories, and national research institutes an unidentified disease in dairy cattle with a short period of clear clinical signs, including fever, decreased milk production, and diarrhea. All classical endemic and emerging viruses, such as pestiviruses, bovine herpesvirus type 1, foot-and-mouth disease virus, bluetongue virus,
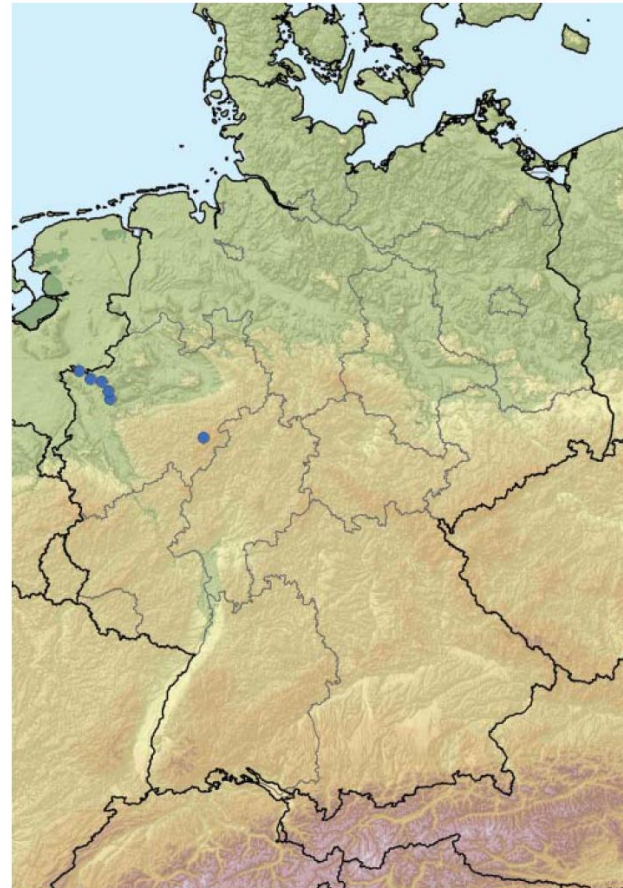

Figure 1. Location of farms with PCR-positive cattle (blue dots) in North Rhine-Westphalia, Germany.
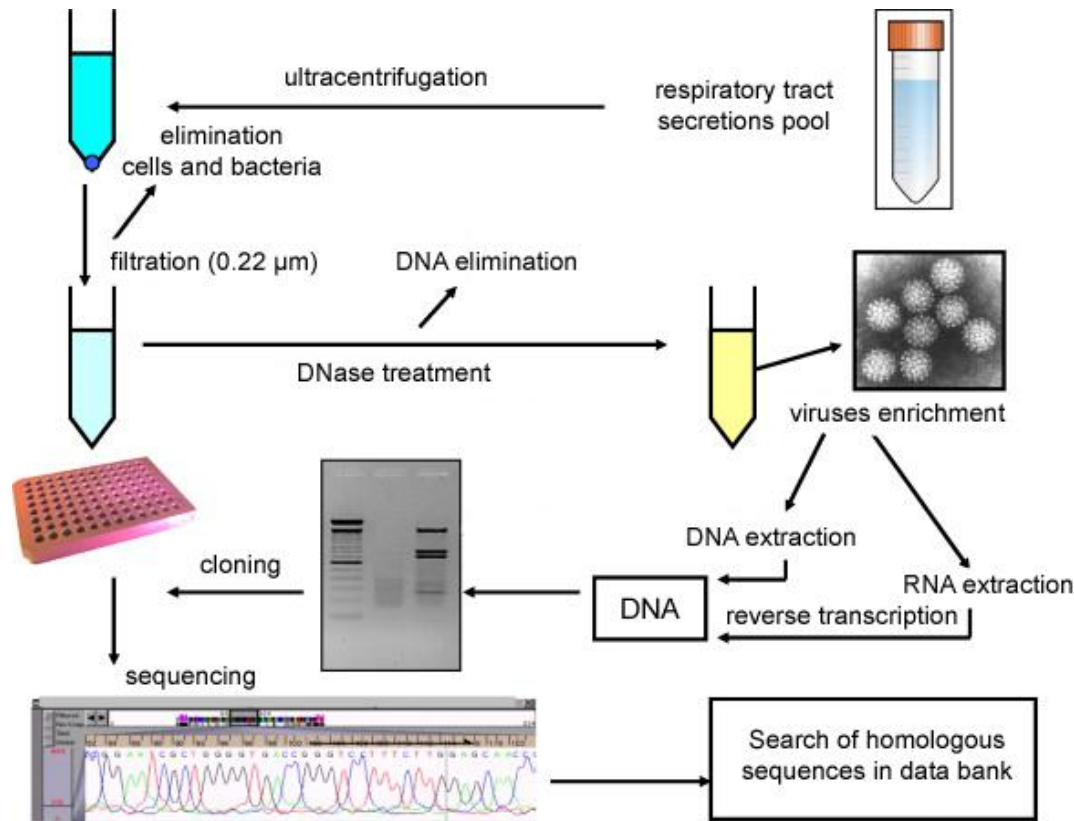
Identification of an unusual syndrome

- *Suspicion of a new introduction of bluetongue*
- *Clinical signs*
  - **fever of over 40°C, reduced general condition, loss of appetite and a drop in milk yield (up to 50 %); over a few days**
- *Exclusion of*
  - **bluetongue virus, epizootic haemorrhagic disease virus, foot-and-mouth disease virus, bovine viral diarrhea virus and other pestiviruses, bovine herpesvirus 1 and other herpesviruses, Rift Valley fever virus and bovine ephemeral fever virus**
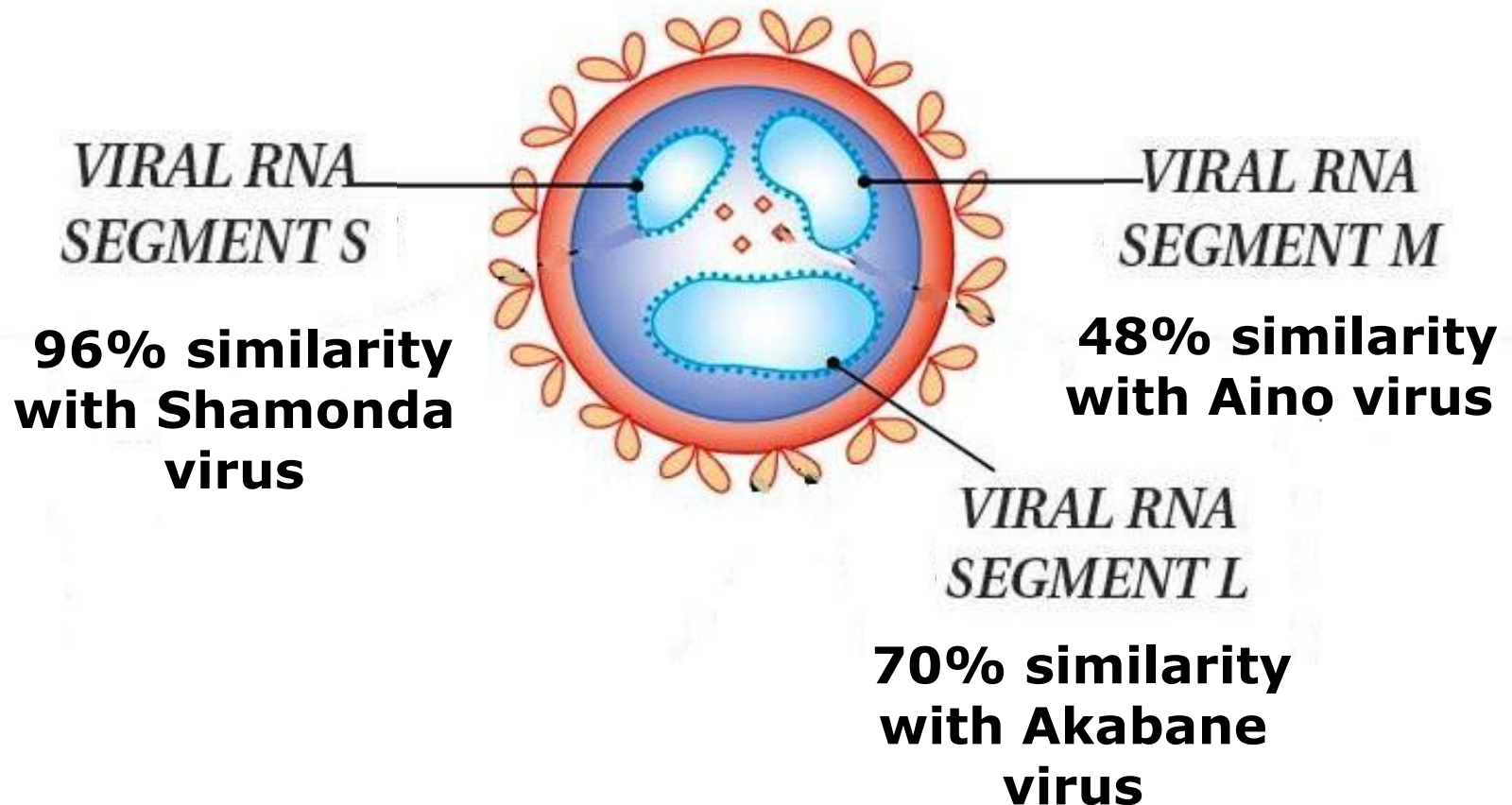 - **No virus observed in infected bovine cell cultures**

Use of refined molecular diagnostic tools

- *« Targeted approach »: Epizone Biochip 5.1 (recognizing more than 2000 viral species)*
  - **Analysis of the Dutch cases**
  - **Fecal samples tested negative**

- *« Random approach »: Metagenomics*
  - **Analysis of German cases**
  - **Blood samples**
  - **Metagenomics protocol**

Discovery of Schmallenberg virus: metagenomics or virus discovery by high throughput sequencing

Schmallenberg virus: genetic relationship with other orthobunyaviruses

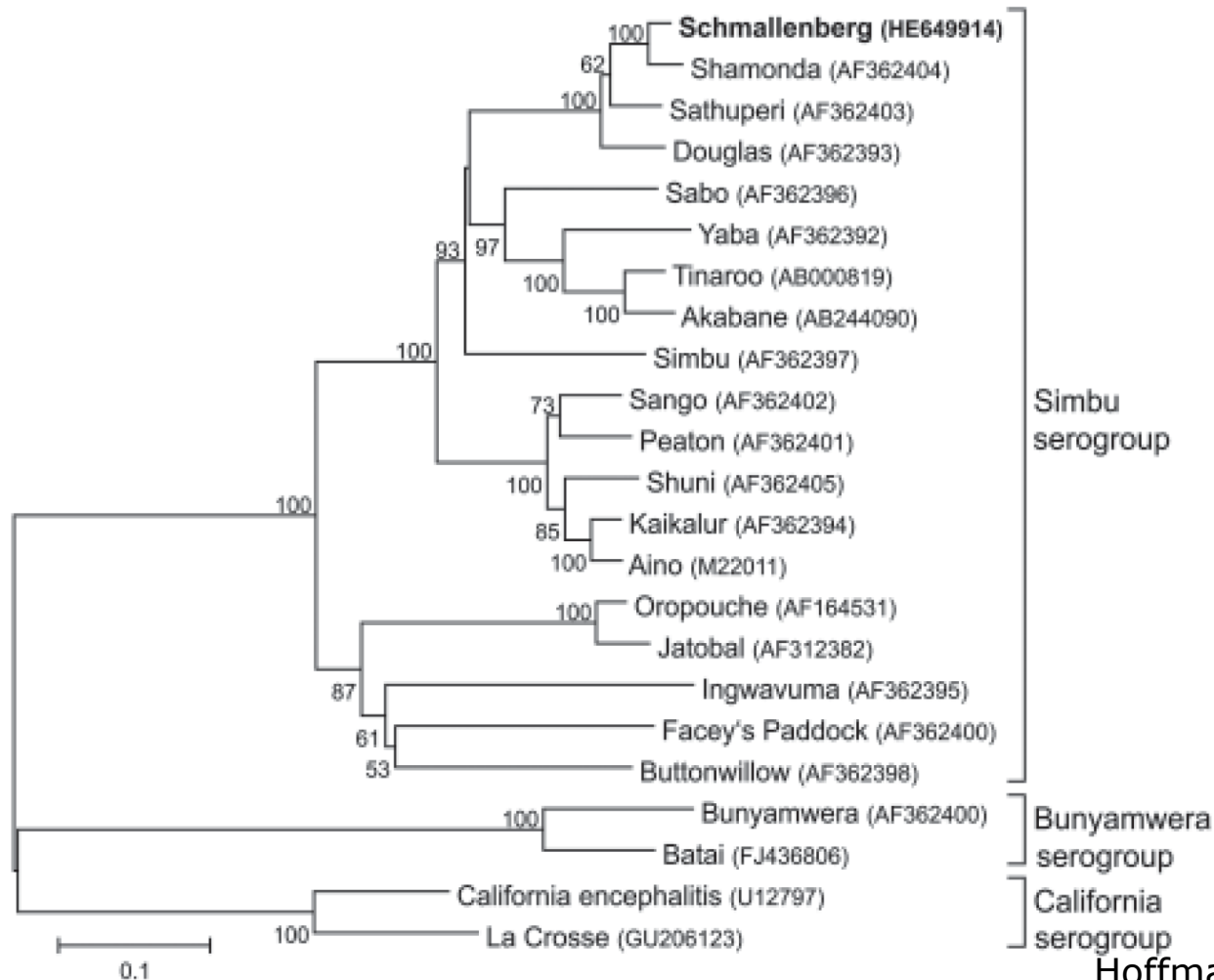VIRAL RNA SEGMENT S

96% similarity with Shamonda virus

VIRAL RNA SEGMENT M

48% similarity with Aino virus

VIRAL RNA SEGMENT L

70% similarity with Akabane virus
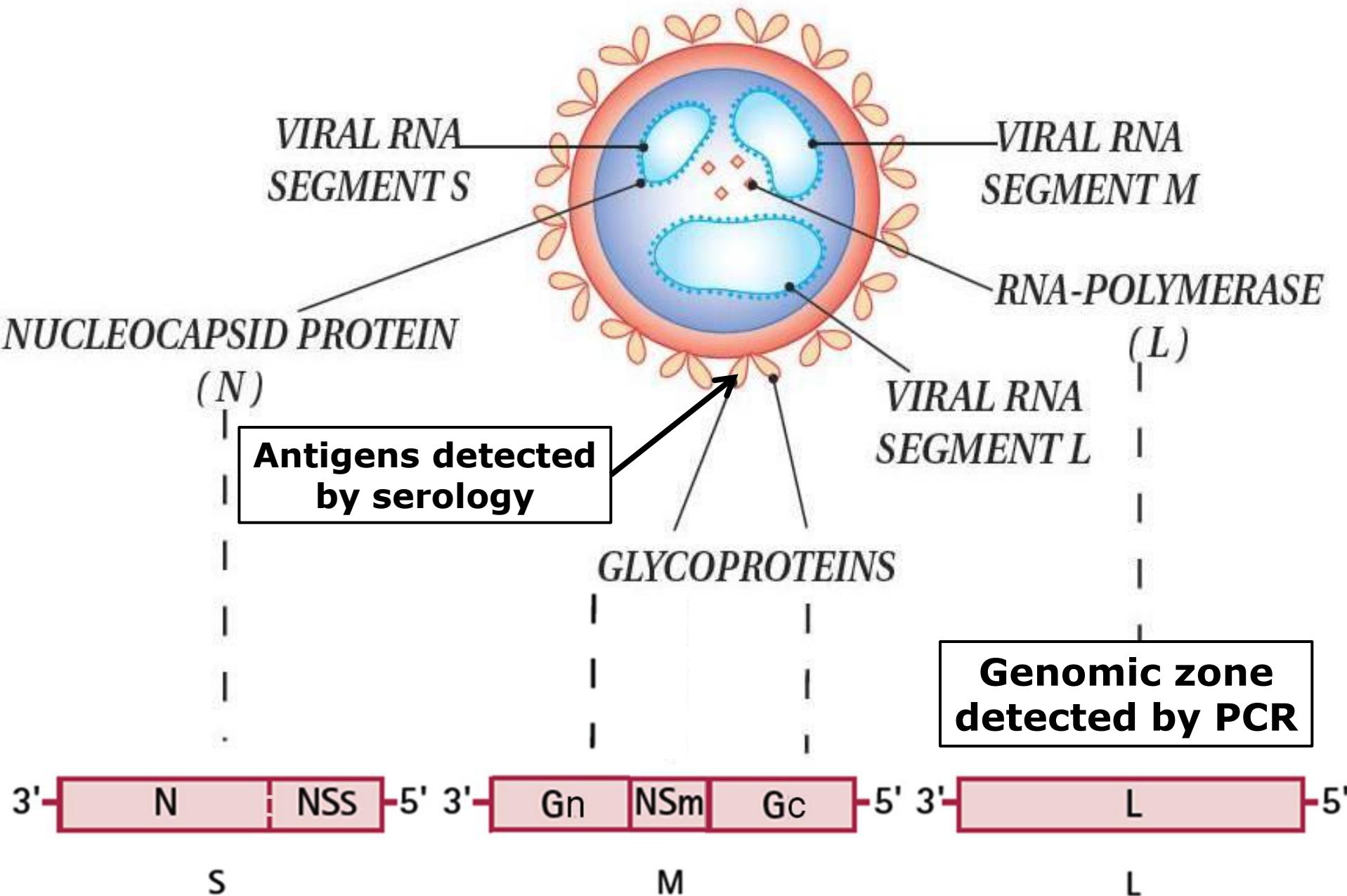
Thiry, 2007

Phylogenetic tree (N gene; S segment)

Hoffmann et al., 2012

Diagnosis of Schmallenberg virus

- *Clinical suspision*

- *Direct diagnosis: virology*
  - **Isolation in cell culture**

    Only experimental
  - **Real-Time PCR on blood**

- *Indirect diagnosis: serology*
  - **Seroneutralisation**

    Good test, but time-consuming and not adapted to large samples
  - **ELISA**

    As for other orthobunyaviruses

    Current setting up: one already validated

VIRAL RNA SEGMENT S

VIRAL RNA SEGMENT M

NUCLEOCAPSID PROTEIN (N)

RNA-POLYMERASE (L)

Antigens detected by serology

VIRAL RNA SEGMENT L

GLYCOPROTEINS

Genomic zone detected by PCR

3'— [N : NSS] —5' 3'— [Gn | NSm | Gc] —5' 3'— [L] —5'

S   M   L

Thiry, 2007

Diagnostic pitfalls

- *In adult*
  - **Subclinical infections – short viremia**
  - **Congenital abnormalities observed late after infection**
- *In fetus/neonate*
  - **Virus well preserved in infected (nervous) tissues and meconium**
  - **Time elapsed between fetus infection and recovery of ill fetus may be long**
  - **Autolysis of fetal tissues: destruction of virus/RNA**
- *Need for a serological tool*
  - **One ELISA test available, no cross-reaction with Akabane virus**
 - **Better suited than seroneutralisation**

Detection of Schmallenberg virus

1. *Passive syndromic vigilance of German and Dutch farmers and veterinarians*

2. *Failure of conventional diagnostic methods*
   - **Non specific (cell culture infection)**
   - **Specific: PCR, etc.**

3. *Failure of multiplex biochips*
   - **Because really new virus**

4. *Success of metagenomics*
   - **Powerful but expensive**
   - **Need for good sample quality**
   - **Need for enough concentration of the infectious agent « above » the background**

Diagnosis
of emerging infectious diseases

- *Syndromic surveillance: farmers and veterinarians*

- *Re-emerging disease: diagnostic available, but few experience of the reference laboratory*

- *Emerging disease: diagnostic approach non specific, broad and « random »*

Atlas of transboundary animal diseases (EN, FR, SP)
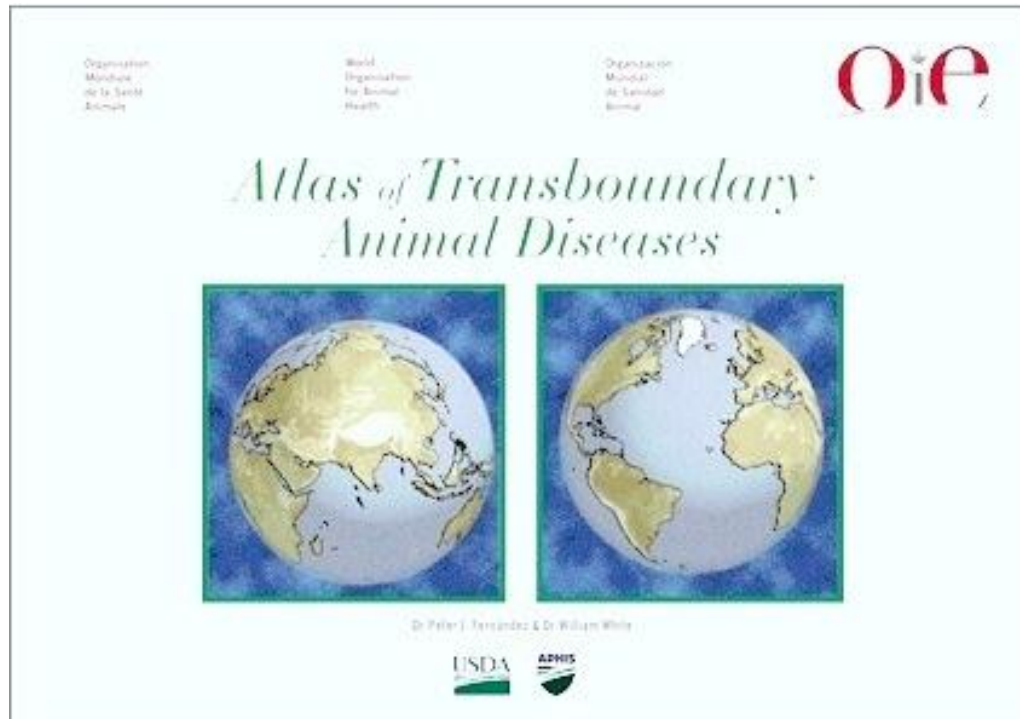
Atlas of transboundary animal diseases

- *Synthetic approach*
- *Description of clinical signs, epidemiology*
- *Atlas of photographs of lesions for the education of the veterinarian on the identification of syndromes*
- *The main 29 infectious diseases at risk of transboundary infection*

Thank you again for your attention