Early diagnosis of animal diseases: a key-element for rapid and efficient management

Dr Pierre Kerkhofs
Veterinary and Agrochemical Research Centre
CODA-CERVA

Emerging animal Diseases: from Science to Policy 17-10-2008
Contents

- What’s an emerging disease?
- Partners of animal disease diagnosis and control
- Lessons from the past
- How to reduce the unforeseeable part of our mission as much as possible?
- Develop advanced expertise in global analysis
- Delegation of certain diagnostic activities to foreign laboratories
- Conclusions
Emerging disease

“An emerging disease is one that has appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range.” (WHO)

- Factors linked to the pathogen and its transmission
- Factors linked to the environment: a disease can be endemic in a region and emerging in another region
- Most (>75%) of human emerging issues are zoonotic

An efficient control of a new outbreak of such a disease depends on many intrinsic factors related to
- the virulence of the germ;
- its contagious power;
- the receptive population;
- the mode of transmission.
Partners in animal disease control

Disease control policy

Veterinary Practitioners

Regional Animal Health Centres
- DGZ-Vlaanderen
- ARSIA

On farm detection, sampling, etc.

Routine diagnostic & dispatching
Animal health experts

FPS Health, Food Chain Safety and Environment

Legislation CVO

National Reference Lab

CODA-CERVA

Reference tasks
Epidemiology

Partners in animal disease control
Belgium notified its first cases of bluetongue on August 18, 2006.

As early as late June, Belgian veterinarians were seeing an unusual number of bovine cases primarily attributed to photosensitisation or exposure to mycotoxins (sporidesmins).

These first cases could not be earlier attributed to bluetongue in the absence of confirmatory tests.
... and in The Netherlands

- Since the beginning of August, a few veterinary practitioners in southern part of The Netherlands observed mandibular oedema and oedema of the lips in a few sheep in sheep flocks and consulted the helpdesk of the Animal Health Service Ltd.
- Further consultations of the helpdesk indicated that these clinical problems were noticed in different sheep flocks at the same time.
- This combination of signals resulted in reporting a suspicion of a contagious disease.
- After the BT suspicion was raised, a rather quick diagnosis was made.
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What are the challenges?

- A crucial step in controlling an outbreak of a new disease is the **early identification of the responsible pathogen**.

- The causal agent **must be identified and completely characterised**
  - in order to be able to evaluate its dissemination capacity
  - to develop additional screening tests and measures for controlling the disease.

- The challenge we are facing with emerging diseases is to **foresee the unforeseeable**, or nearly so.
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To reduce the unforeseeable part of our mission

- Thorough knowledge of the **worldwide epidemiological situation and its evolution**
  - to optimally foresee the potential introduction and outbreak of an emerging agent.

- CODA-CERVA is collaborating in an **international network of reference laboratories**
  - monitoring the world animal health situation;
  - deciding when additional investments are needed to prepare for emerging diseases.

- Epidemiologists provide crucial information to the reference laboratories through **risk analyses**
  - climate changes;
  - estimation of disease prevalence;
  - developments in trade, transport;
  - land use.
The clinical observation of farm animals by the farmers and the veterinarians is the key element of a rapid detection.

All these individual observations are to be collected and analysed to detect trends in animal diseases in a region.

- Help desk (AHS, The Netherlands)
- Information systems
  - RADAR (Defra);
  - Emergence (INRA);
  - Veterinary Practitioner Aided Disease Surveillance (New Zealand);
  - Rapid Syndrome Validation Project—Animal (USA).
Brings together key surveillance information collected in other systems about animal diseases and conditions in a structured and consistent way.

Contains current, accurate information about the number and location of animals.

Will allow earlier detection of threats by:
- harmonising and quality tagging data collection
- Prioritising, streamlining data analysis
- improving dissemination...

The strategy proposes a surveillance framework based on:
- Strategic Goal 1: Collaboration
- Strategic Goal 2: Prioritisation
- Strategic Goal 3: Derive better value from raw data
- Strategic Goal 4: Sharing information more widely
- Strategic Goal 5: Quality Assurance
Central theme in this strategy: collaboration

- **Collaborative working is essential** to enable successful implementation-avoiding gaps / duplications, building consensus & wide ownership of the approach.

- **Achieved through:**
  - Formal & ad hoc consultation with stakeholders
  - Planned commitment of the appropriate people at the right time
  - Governance arrangements involving stakeholders (Business Assurance Groups)
  - Range of communication channels e.g. the “Vet Surveillance in the UK” Website at www.defra.gov.uk/animalh/diseases/vetsurveillance/index.htm
RADAR facilitates

- Earlier detection / intervention of new & emerging diseases
- Improved chance of detecting links between human & animal disease
- More effective targeting of resources for surveillance & control
- Policy making will be based on improved evidence of disease risk and impact
- Better informed stakeholders who are better able to identify, understand and communicate animal-associated threats
- Consumers and animal owners making more informed decisions at a personal level
The approach focuses on detecting individual atypical cases.

Based on how previous emerging diseases have been detected, atypical cases can arise from:

- a new disease that shows clinical signs the clinician cannot link to a known disease;
- a known disease expressed atypically through unusual clinical signs, atypical region or species, or increased severity;
- a rare or inadequately documented sporadic disease.
Notification of atypical syndrome

- Initial notification and automatic follow-up
- Compromise between precision - ease of use
- 3 geographic levels (country, department, region)
- Automatic alert of experts after classification
Info and real-time statistics

**émergences.**

**NOTIFICATIONS ET STATISTIQUES CONCERNANT L’ENSEMBLE DES NOTIFICATEURS**


**NOTIFICATIONS**

- Notifications effectuées au cours des 30 derniers jours
- Notifications par pays, période, espèce et maladie

**STATISTIQUES DESCRIPTIVES : NOTIFICATIONS AVEC SUVI**

- Nombre de notifications trimestrielles par localisation, année, espèce et maladie
- Pourcentages de clients/organismes ayant notifié par pays, année, trimestre, espèce et maladie
- Taux de mortalité, de morbidité, et de latence par pays, année, espèce, filière de production et maladie
  - catégorie la plus atteinte

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**NOTIFICATIONS ET STATISTIQUES GÉNÉRALES**

Cas notifiés au cours des 30 derniers jours en France pour l’espèce Bovine

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**Retour** | **Sommaire**
Dynamic geographical analysis
Limitations of systems based on clinical observation

- Atypical case detection is limited by practitioners’ experience, scientific knowledge, vigilance, and willingness to report findings.
  - Multiple, similar reports of atypical cases improve confidence that a new disease is emerging.
  - Vigilance should be enhanced by specific training courses.

- A clinical reporting tool alone is only the first step to determine if the cases share an etiologic pathway.
  - Review by expert clinicians, necropsy findings, immunologic screenings, and focused epidemiologic studies play key roles in such determination.

- The need to establish baseline levels for defined syndromes.
  - Requires time and resources;
  - Is common responsibility of all involved in animal diseases;
  - Without them, we cannot know when the incidence of a syndrome has significantly increased.
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Global analyses

“All catching techniques”

- Necropsy, anatomo-pathology and histology
- Electron microscopy (EM)
- Molecular techniques
  - Micro-arrays
  - Random sequencing
Resolving power of EM
## Diagnosis by EM

<table>
<thead>
<tr>
<th>Argument (Why)</th>
<th>Importance (When)</th>
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<tr>
<td>- Non-specific</td>
<td>- Specific test fail</td>
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<tr>
<td>- Non-directed</td>
<td>- Specific tests are unavailable</td>
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<tr>
<td>- Open-view</td>
<td>- Pathogens are new, rare, changed</td>
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<tr>
<td>- No a priori choice of pathogen</td>
<td>- Pathology / question is complex</td>
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<tr>
<td>- (Can be) rapid</td>
<td>- Orientation required</td>
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<tr>
<td>- Independent confirmation</td>
<td>- The situation is urgent</td>
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<tr>
<td>- Morphology-based confirmation</td>
<td>- People are impatient, curious</td>
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<td></td>
<td>- To avoid / end discussions</td>
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<tr>
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<td>- “Pictures don’t lie”</td>
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<td></td>
<td>- “One image is worth a thousand words”</td>
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<td></td>
<td>- To help people better</td>
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</table>
EM for diagnosis of emerging diseases

- Heterogeneous and **multiple pathogens** can be detected
- Orientation of ‘rare, difficult, unknown’ syndromes or pathogens
- Integral part of the routine laboratory diagnosis, can be done **before, or in parallel** with, specific tests
  - Independent confirmation and control of molecular test results
  - Quality assurance of results and assays
- Limitations
- **Combination of EM and nucleic acid amplification techniques** (NAT, Real time PCR) executed in parallel
  - EM: rapid risk assessment (herpes virus = no action, orthopox: alert)
  - NAT: confirmation
Problem

- Several outbreaks of viral zoonoses (SARS, monkey pox USA, Avian influenza, Hendra virus, West Nile disease, ...)

- Each time, EM was important:
  - As a first-line test, for orientating diagnosis (no need of specific reagents)
  - For independent confirmation of other test results
  - To characterise the morphology and morphogenesis of the virus

- EM will certainly be used in a next crisis.

- For specific virus families containing candidate new, emerging and rare virus, insufficient expertise of morphology and morphogenesis is available.
Objectives

- (Maintain and develop expertise – the only unit in Belgium)
- **Pro-active characterisation** of candidate emerging diseases.
  - Using standard and advanced EM techniques
    - Negative staining
    - Ultra-thin sectioning
    - Immuno-EM [Immunogold NDV](#)
    - Cryo-EM
    - Electron tomography
- Well-chosen models of specific virus families
Urgent differential diagnosis
FMDV - Parapoxvirus
EM confirmation of BTV-8 in Belgium

First BTV-8 virion observed in Europe (bluetongue crisis 2006)

Characteristic micrographs of BTV-8
Microarray technology

- High-throughput technology
- Arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing picomoles of a specific DNA sequence
  - short section of a gene;
  - other DNA element

- Probe-target hybridization is detected and quantified by fluorescence-based detection of fluorophore-labeled targets to determine relative abundance of nucleic acid sequences in the target.
DNA microarray for BSL-3 agents detection

**PHILOSOPHY** : 1 suspect sample, 1 test for all agents ...

**Array Format** : PAMGENE™
- Fluorescence detection, liquids are flushed through
- Single tube technology (multiplexed hybridization reactions)

**Subcontracting partner** : Check-Points™ (Wageningen, NL)

Initial probe design for 7 BSL-3 bacteria and 4 animal viruses
### First results

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<th>DNA source</th>
<th>conc. (ng) assay</th>
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<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
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¹Qiagen prep's (QG DNA Easy kit)
²Plasmid miniprep + restrictie + EtOH precipitation
Evaluation of PAMGENE format

- **Advantages**
  - few DNA quantity needed (10^{-12} g)
  - few amplified PCR product needed (1-50 10^{-9} g)
  - excellent specificity (at SNP level)
  - single-tube processing
  - flush processing of the array (allows T° adjust. and automatization)

- **Disadvantages**
  - LDR lasts min. 10 hours (improvable)
  - Limited array capacity (± 100 spots)
  - Limited sensitivity
DNA sequencing encompasses biochemical methods for determining the order of the nucleotides bases G A T C in a DNA sequence.

The sequence of DNA constitutes the heritable genetic information in nuclei, plasmids, virus, mitochondria, and chloroplasts that forms the basis for the developmental programs of all living organisms.

High-throughput sequencing technologies reduce the cost of sequencing DNA libraries beyond what is possible with DNA separation by capillary electrophoresis.

Many of the new high-throughput methods use methods that parallelize the sequencing process, producing thousands or millions of sequences at once.

These technologies enable amplification and identification of randomly selected nucleotidic sequences in an unknown sample.
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How to face all animal infectious diseases?

- Questions and problems concerning all transmissible animal diseases can be posed to CODA-CERVA.

- Due to the restricted financial means allocated for this mission, CODA-CERVA doesn’t have complete competency in house for all these diseases. Even if scientists are able to answer to questions, laboratory analyses cannot be performed for all these diseases.

- This entire field has to be covered by networking with European laboratories;
- Confirmation diagnosis is performed by the CRL;
- Close collaboration with specialized laboratories enables CODA-CERVA to guarantee the authorities a diagnosis of almost all transmittable diseases of animals.
How to face all animal infectious diseases?

- Depending on the risk analysis of the appearance of a disease in Belgium, it is to be decided whether:
  - the entire diagnosis is delegated abroad (Rinderpest, Peste des petits ruminants,...);
  - the first-line tests are conducted at CODA-CERVA; a foreign reference laboratory is involved for the confirmation tests (AHS, ASF, WNV,...);
  - the entire diagnosis is carried out at CODA-CERVA (epizootic and enzootic diseases).

- To guarantee a satisfactory diagnostic response time to the authorities, the collaboration with the outsource laboratory is established on a contractual basis.
Bluetongue diagnosis

- **Before 2004:**
  - No laboratory diagnosis performed at CODA-CERVA;
  - Analyses outsourced in AFSSA.
- **2004:**
  - Modification of the import risk analysis (climate change);
  - Implementation of first line techniques, confirmation in AFSSA;
  - Launch research programmes in collaboration with AFSSA.
- **2006:**
  - All the diagnostic techniques are carried out at CODA-CERVA;
  - A new RT-PCR method is developed in collaboration with AFSSA;
  - A collaboration agreement is signed by both directorate general of AFSSA and CODA-CERVA;
  - CODA-CERVA is the first to isolate and identify BTV-8 in northern Europe.
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To face successfully the next emerging disease

- we need:
  - a **rapid identification** of the new disease and pathogen;
  - an efficient **collaborative network** between epidemiologists; microbiologists, entomologists and risk managers
  - a **control program** elaborated using the results of the risk assessment.

- **Early detection, in the field and at the laboratory, is one of the major keys to its successful control.**
Acknowledgments

All the colleagues in CODA-CERVA who are ready - working to be ready - for the diagnosis of an emerging disease.

- MOSS: M. Dispas, C. Herr
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- Microarrays: P. Wattiau, F. Vandenbussche
- DNA sequencing: S. van Borm
- The reference laboratories for their active collaboration with foreign laboratories
- The epidemiologists of the CCVD


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