



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

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Dr Pierre Kerkhofs  
Veterinary and Agrochemical Research Centre  
CODA-CERVA

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# 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





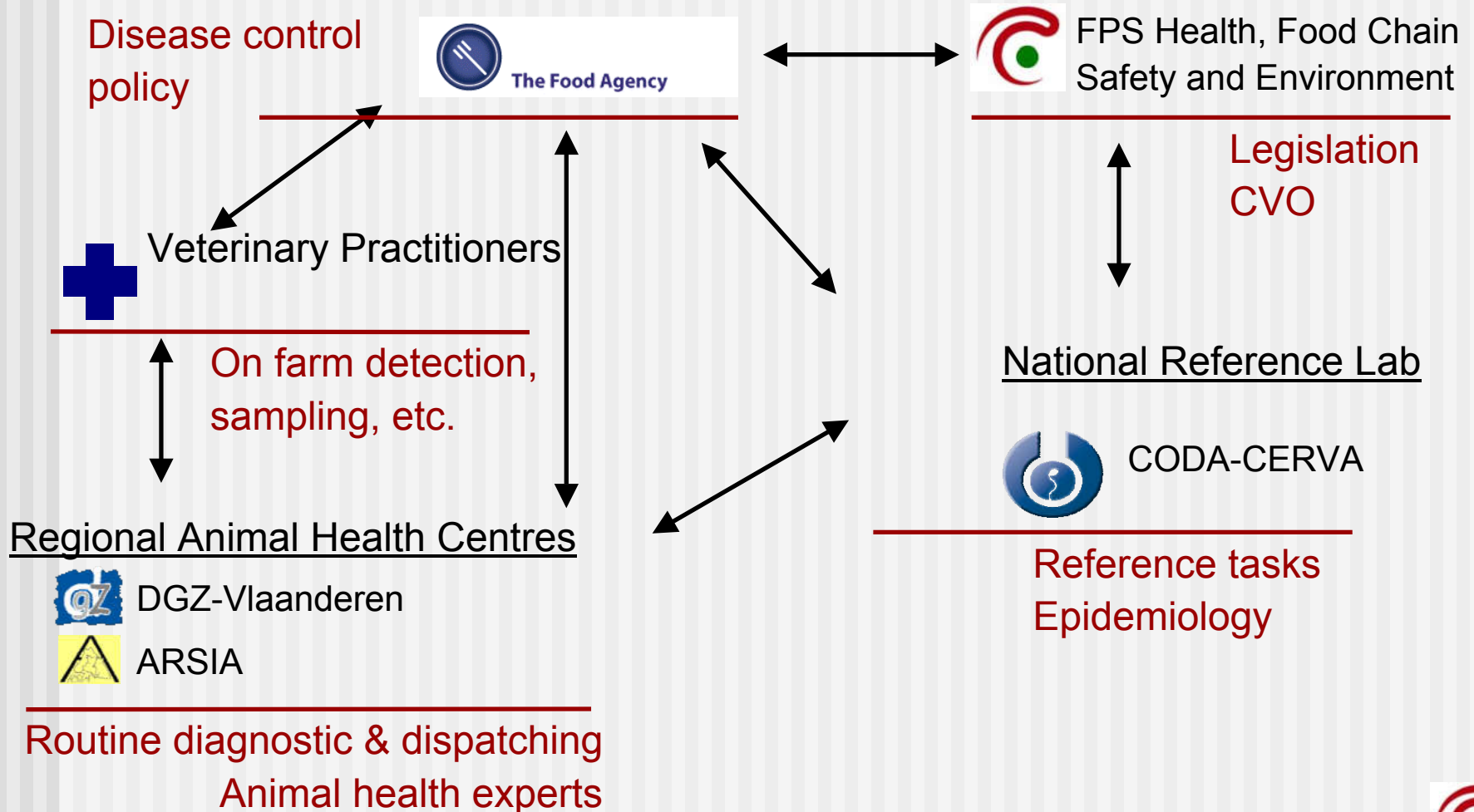
# Emerging disease

- “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





# Most recent example: Belgium

- 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**.



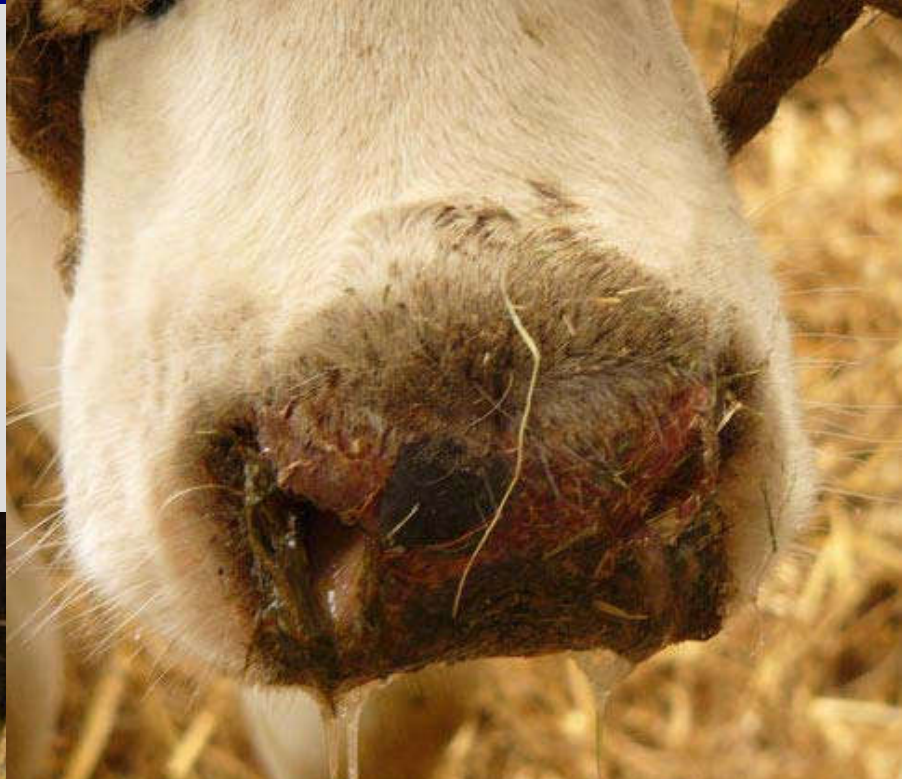
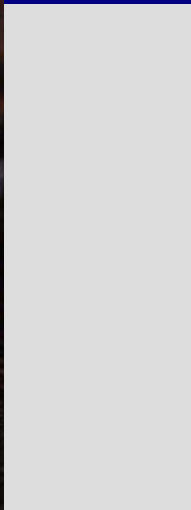


## ... and in The Netherlands

- 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.





# Rapid knowledge of the epidemiological evolution

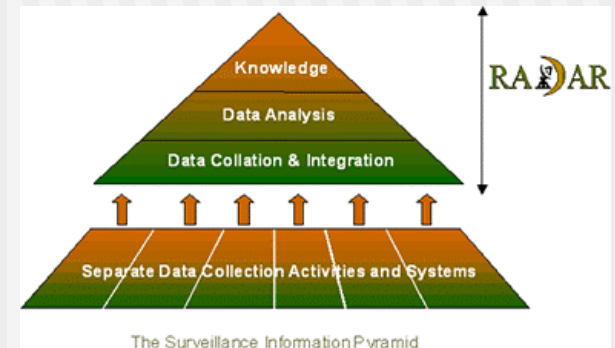
- The **clinical observation** of farm animals by the farmers and the veterinarians is the key element of a rapid detection.
- All these individual observations are to be collected and analysed to detect trends in animal diseases in a region.
  - Help desk (AHS, The Netherlands)
  - Information systems
    - **RADAR (Defra);**
    - **Emergence (INRA);**
    - **Veterinary Practitioner Aided Disease Surveillance (New Zealand);**
    - **Rapid Syndrome Validation Project—Animal (USA).**





# RADAR - Defra

- 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](http://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







# Emergences - INRA

- 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

NOTIFICATION D'UN SYNDROME ATYPIQUE

Notification n° 3555

Le cas est-il :  observé par vous ?  observé par un autre vétérinaire (y compris de votre cabinet) ?  rapporté par un éleveur ?

Espèce concernée : Bovine

Date d'apparition des premiers symptômes : Jour : 30 Mois : Août Année : 2006

Effectif total des animaux de l'espèce dans l'élevage : 0

Orientation de l'élevage : Choisir SVP

Syndrome déjà mentionné par vous comme atypique : Ne sais pas

\* Elevage = lieu d'entretien d'un ou plusieurs animaux à des fins économiques ou de plaisir

Localisation de l'élevage

Pays : FRANCE

Localisation : AIN

Ville : AMAREINS

Code de l'élevage :

Code de l'élevage = Les quatre premières lettres du nom du responsable de l'élevage, suivies des quatre premières lettres de son prénom (NB: "Code de l'élevage" commandé à "Numéro/nom" sur l'application PC).

Tableau clinique

Éléments ayant conduit à la notification

Observation d'un signe clinique très particulier ou bizarre :

Tableau clinique non attribué à une maladie répertoriée :

Expression atypique d'une maladie répertoriée :

- Nom de la maladie :

- Maladie non connue ou rare dans la région :

- Maladie non connue ou rare dans l'espèce :

- Gravité exceptionnelle de la maladie :

- Aucune réponse à un traitement normalement très efficace :

Autre atypicité :

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

Description précise du tableau clinique du syndrome (obligatoire) :

Effectifs estimés des catégories animales les plus atteintes à ce jour par le syndrome au sein de l'élevage (la ou les catégories les plus atteintes, ainsi que la race la plus atteinte, sont celles qui correspondent au plus grand nombre de malades)

Race la plus atteinte : Indéterminée

Catégorie la plus atteinte : Indéterminée

- Nombre de malades : 0

- Nombre de morts parmi les malades : 0

- Nombre total d'animaux de la catégorie dans l'élevage : 0

Secundo catégorie la plus atteinte : Indéterminée

- Nombre de malades : 0

- Nombre de morts parmi les malades : 0

- Nombre total d'animaux de la catégorie dans l'élevage : 0

Autres catégories atteintes de l'espèce : Bovine

- Nombre de malades : 0

- Nombre de morts parmi les malades : 0

Renseignements concernant la catégorie la plus atteinte au sein de l'élevage

Appareils / systèmes :

Principalement atteint : Aucun

Signes d'hémorragie :

Autre appareil atteint : Aucun

Signes d'hémorragie :

Autre appareil atteint : Aucun

Signes d'hémorragie :

Autre appareil atteint : Aucun

Signes d'hémorragie :

Autre appareil atteint : Aucun

Signes d'hémorragie :

Autre appareil atteint : Aucun

Signes d'hémorragie :

Autres caractéristiques cliniques / thérapeutiques :

Température corporelle moyenne : Indéterminée

Durée moyenne d'évolution du syndrome : Indéterminée

A quel moment de la vie se manifeste principalement le syndrome : Indéterminé

Par rapport à la mise bas (si pertinent), quand se manifeste principalement le syndrome : Sans objet

Traitement mis en oeuvre (molécules) :

Contact avec d'autres animaux

Te ne les premiers animaux atteints ont-ils été depuis moins d'un mois ? (choix multiple possible)

- Introduits dans l'élevage :

- au contact d'animaux de même espèce introduits dans l'élevage :

- au contact d'animaux de même espèce extérieurs à l'élevage (marché, concours) :

- information inconnue :

Annuler Valider

Dernière connexion : 29/03/2006 16:06:47  
Tentatives échouées : 0  
Vieillesse : 15344

Inscription Vos notifications Vos enquêtes Résultats généraux Aide

Téléchargement des mises à jour

Menton, Hauts, Mios à jour le 30 Février 2006



# Info and real-time statistics

**INRA émergences.**

accueil  
partenaires  
recherches  
actualités  
liens  
contact  
..... identité  
Mme ESSAÏ *essaï01*  
ST GENES CHAMPANELLE  
>> Résultats généraux  
organisation système  
> notifications et statistiques générales  
cartographie  
enquêtes réalisées  
forum  
bulletin du site  
liste des collaborateurs

Dernière connexion : 21/05/2007 16:25:35  
Tentatives échouées : 0

Visiteurs : ?

Inscription Vos notifications Vos enquêtes Résultats généraux Aide

Téléchargement des mises à jour

Mention légale Mise à jour le 10 Février 2006

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

**i** Précision : dans les tableaux statistiques, l'année commence le 1er décembre précédant l'année choisie et s'achève le 30 novembre de l'année choisie (l'année va donc du 1/12/2004 au 30/11/2005, si l'année choisie est 2005)

**NOTIFICATIONS**

Liste des notifications

- **Notifications effectuées au cours des 30 derniers jours**

Consultation des notifications avec suivi

- **Notifications par pays, période, espèce et maladie**

**STATISTIQUES DESCRIPTIVES : NOTIFICATIONS AVEC SUIVI**

- **Nombre de notifications trimestrielles par localisation, année, espèce et maladie**
- **Pourcentages de clientèles / d'organismes ayant notifié par pays, année, trimestre, espèce et maladie**
- **Taux de mortalité, de morbidité, et de létalité par pays, année, espèce, filière de production et maladie :**
  - **toutes catégories atteintes**
  - **catégorie la plus atteinte**

**émergences.**

**NOTIFICATIONS ET STATISTIQUES GÉNÉRALES**

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

N°	Date	Localisation	Type de maladie	Nom
▼▲	▼▲	▼▲	▼▲	▼▲
I-3745	23/04/2007	ALLIER	Piroplasmose	<b>ROUMEGOUS</b>
I-3746	24/04/2007	ALLIER	Ehrlichiose	<b>MATHIS</b>
I-3749	25/04/2007	ALLIER	Syndrome atypique	<b>THIERCY</b>
I-3752	05/05/2007	ALLIER	Piroplasmose	<b>FINCK</b>
I-3753	05/05/2007	ALLIER	Syndrome atypique	<b>FINCK</b>
I-3754	05/05/2007	ALLIER	Syndrome atypique	<b>FINCK</b>
I-3755	05/05/2007	ALLIER	Syndrome atypique	<b>FINCK</b>
I-3757	05/05/2007	ALLIER	Syndrome atypique	<b>MATHIS</b>
I-3758	07/05/2007	ALLIER	Piroplasmose	<b>PERRIN</b>
I-3762	11/05/2007	ALLIER	Ehrlichiose	<b>CHANTREAU</b>

Retour Sommaire

Dernière connexion : 21/05/2007 16:25:35  
Tentatives échouées : 0

Visiteurs : ?

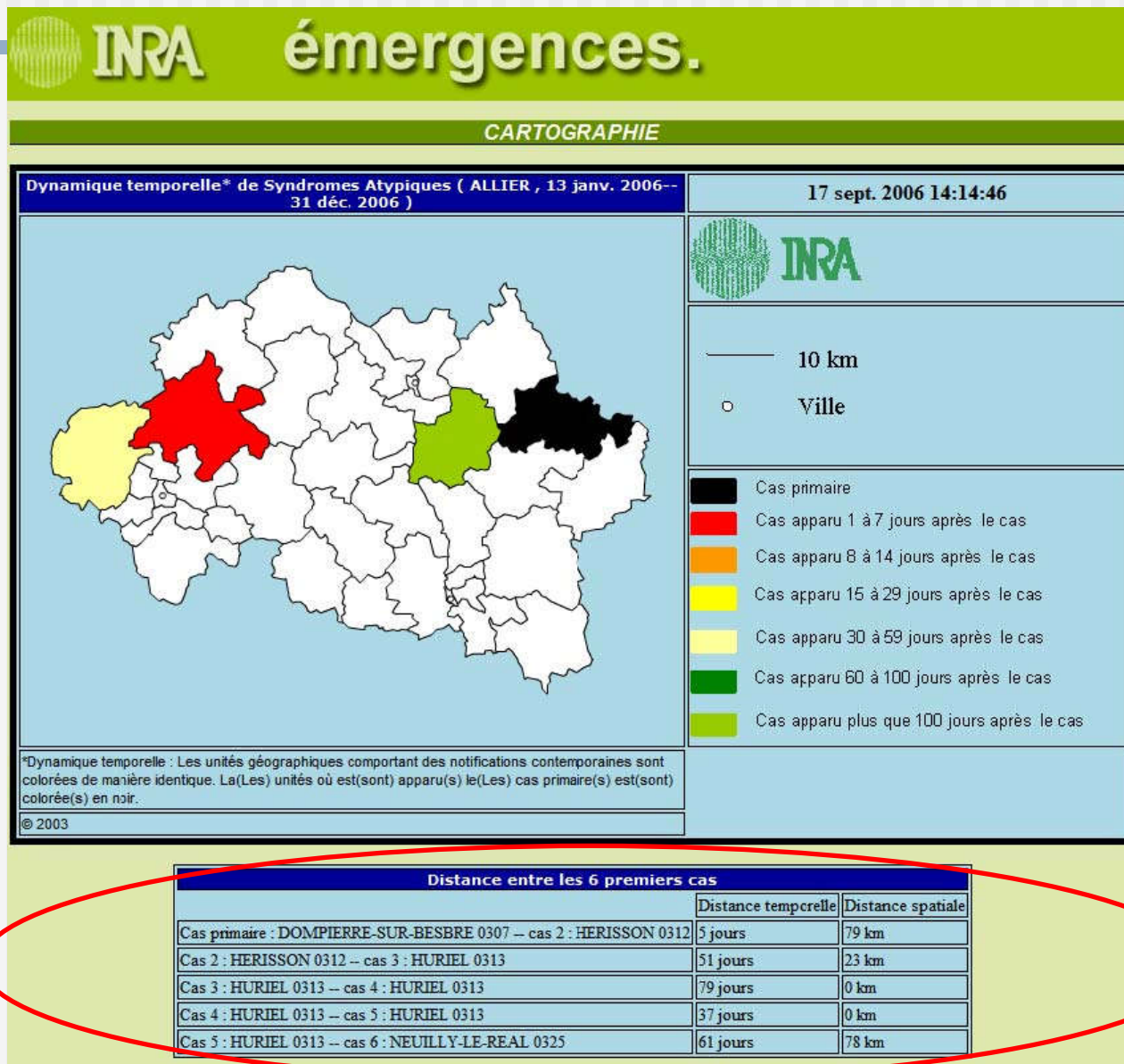
Inscription Vos notifications Vos enquêtes Résultats généraux Aide

Téléchargement des mises à jour

Mention légale Mise à jour le 10 Février 2006



# 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

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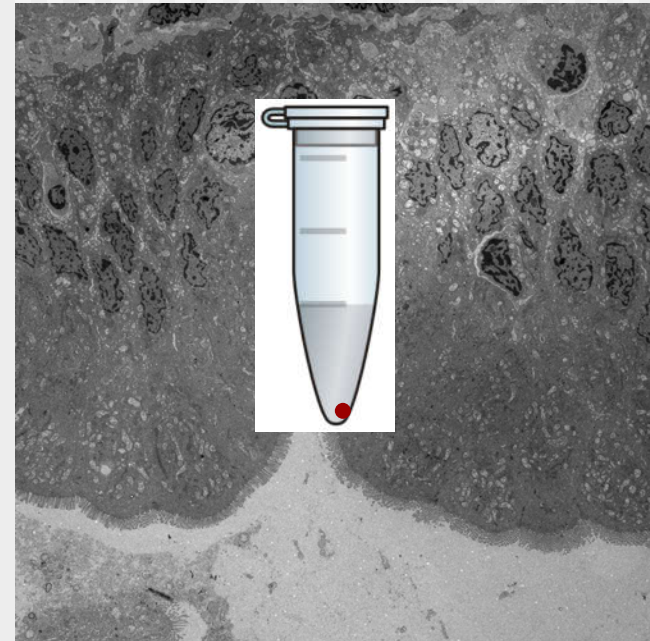
## “All catching techniques”

- Necropsy, anatomo-pathology and histology
- Electron microscopy (EM)
- Molecular techniques
  - Micro-arrays
  - Random sequencing





# Resolving power of EM



300000  
250000  
150000  
120000  
98000  
68000  
49000  
30000  
23000  
18500  
13000  
9300  
6800  
4800  
2900  
1900  
1400  
1200  
890  
690  
1





# Diagnosis by EM

Argument (Why)	Importance (When)
<ul style="list-style-type: none"><li>■ <b>Non-specific</b></li><li>■ <b>Non-directed</b></li><li>■ <b>Open-view</b></li><li>■ <b>No a priori choice of pathogen</b></li></ul>	<ul style="list-style-type: none"><li>■ <b>Specific test fail</b></li><li>■ <b>Specific tests are unavailable</b></li><li>■ <b>Pathogens are new, rare, changed</b></li><li>■ <b>Pathology / question is complex</b></li><li>■ <b>Orientation required</b></li></ul>
<ul style="list-style-type: none"><li>■ <b>(Can be) rapid</b></li></ul>	<ul style="list-style-type: none"><li>■ <b>The situation is urgent</b></li><li>■ <b>People are impatient, curious</b></li></ul>
<ul style="list-style-type: none"><li>■ <b>Independent confirmation</b></li><li>■ <b>Morphology-based confirmation</b></li></ul>	<ul style="list-style-type: none"><li>■ <b>To avoid / end discussions</b></li><li>■ <b>“Pictures don’t lie”</b></li><li>■ <b>“One image is worth a thousand words”</b></li><li>■ <b>To help people better</b></li></ul>



# 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
  - o EM: rapid risk assessment (herpes virus = no action, orthopox: alert)
  - o 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.





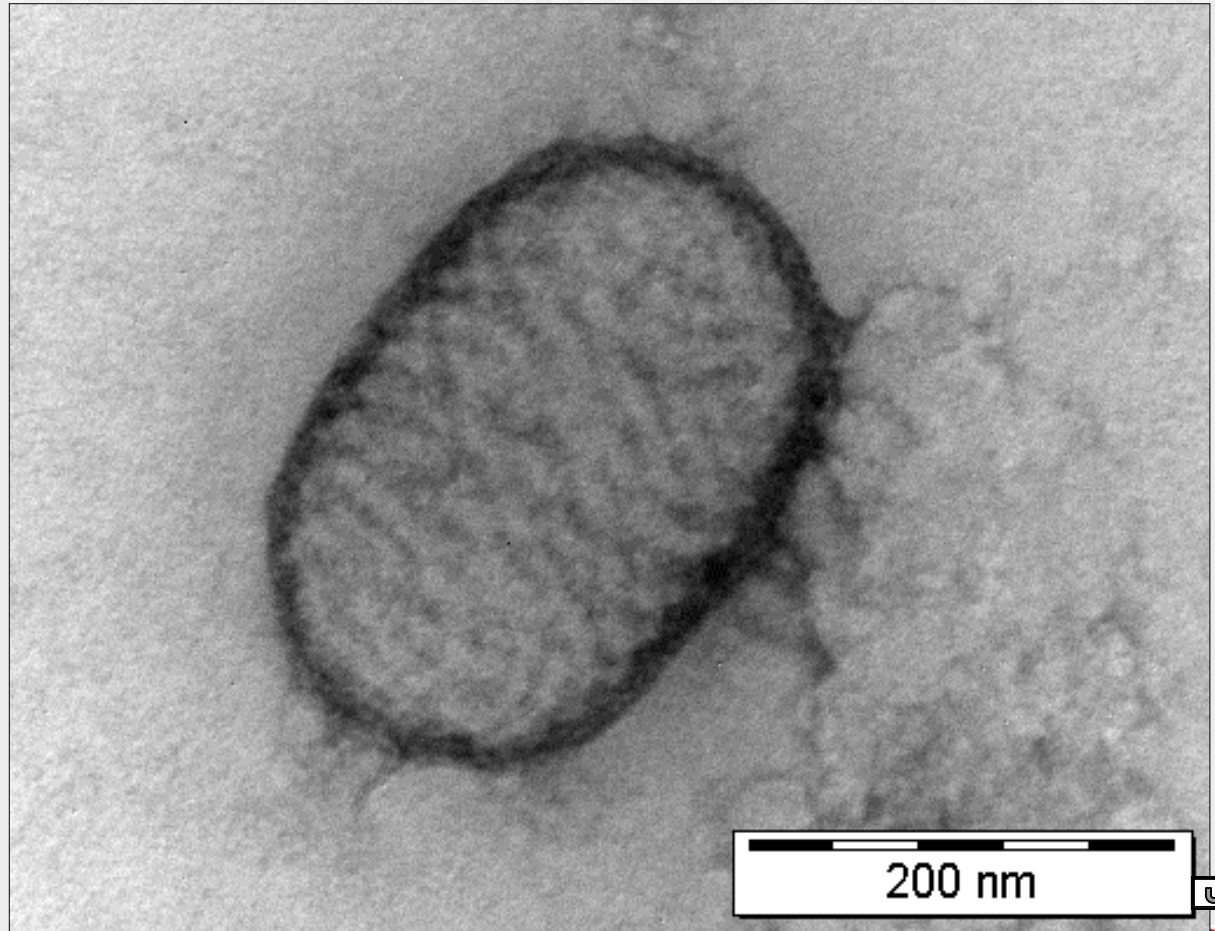
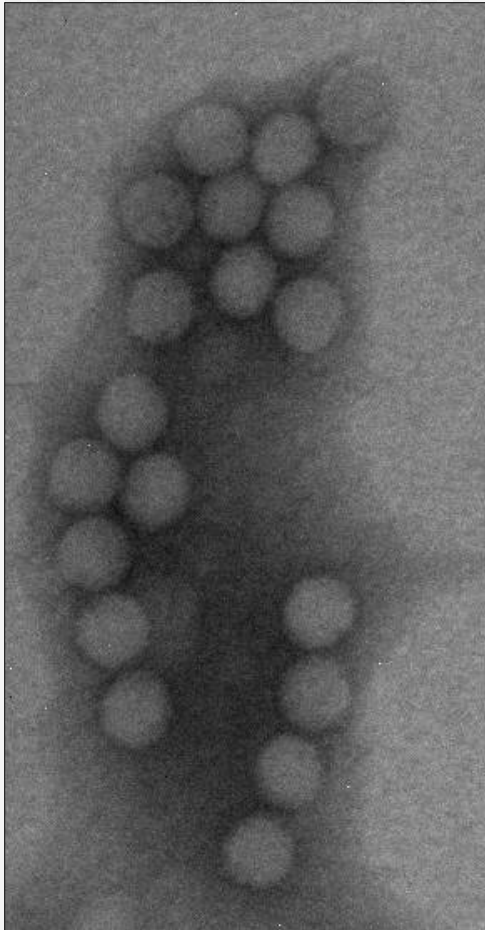
# Objectives

- (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



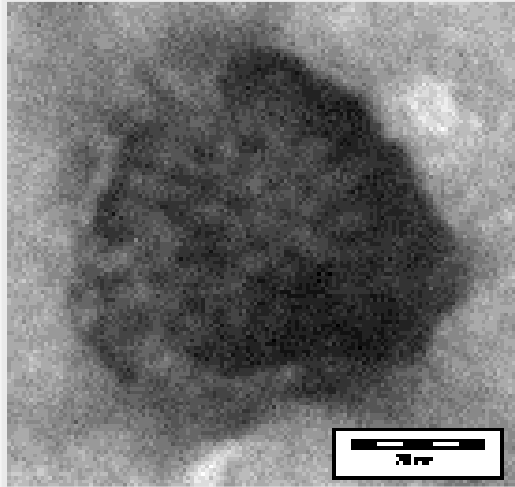


# Urgent differential diagnosis FMDV - Parapoxvirus

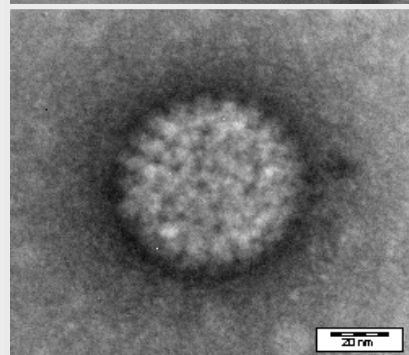
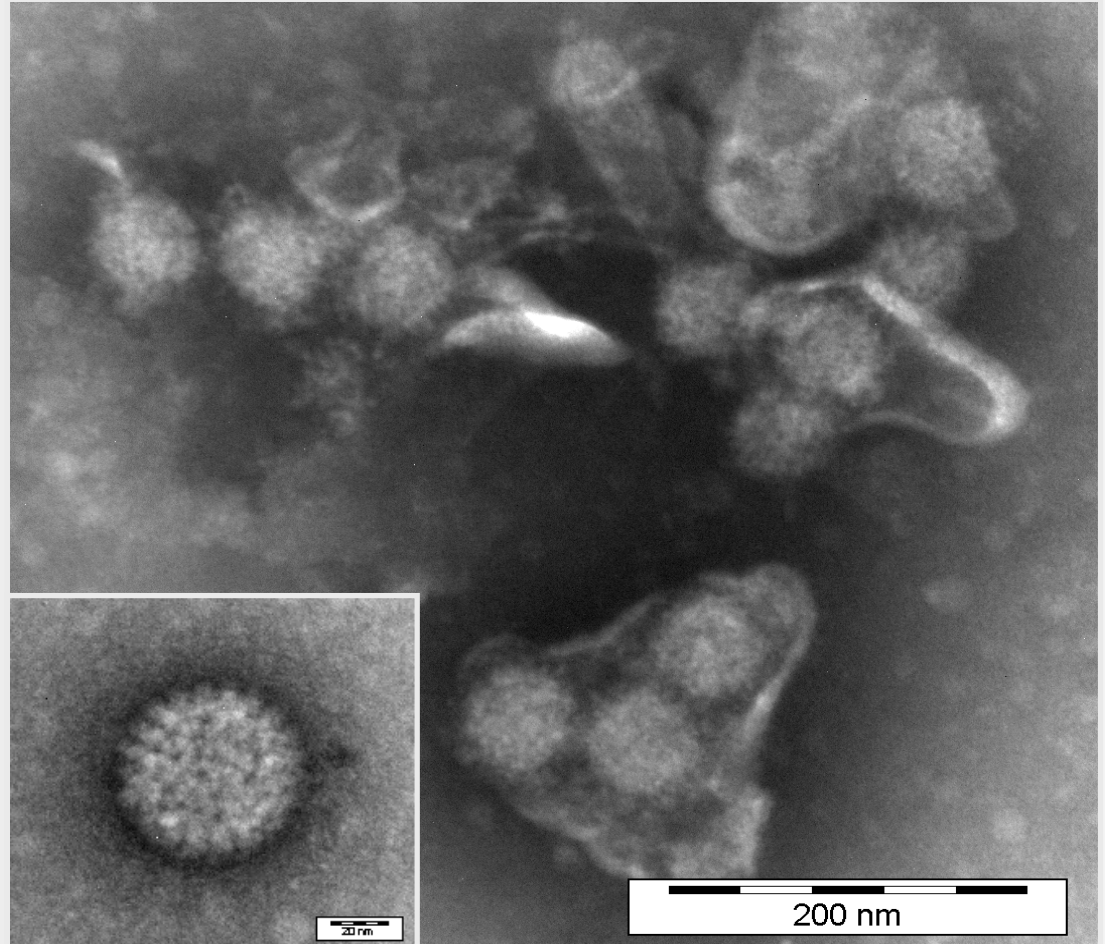




# EM confirmation of BTV-8 in Belgium



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



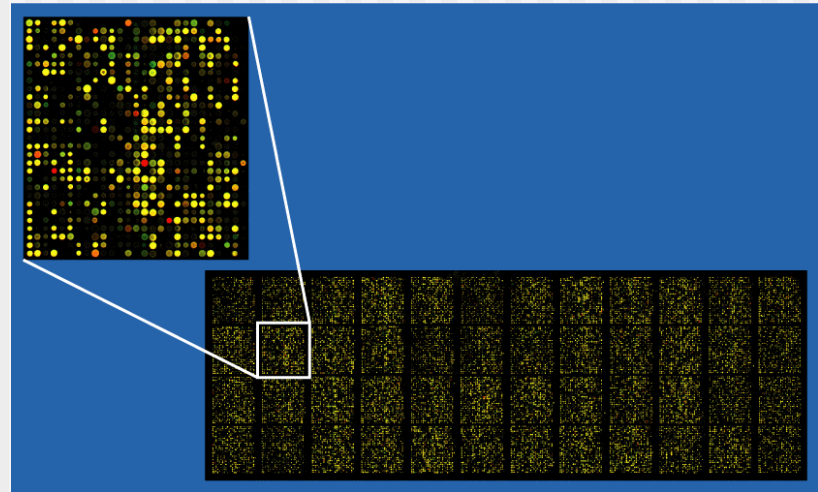
**Characteristic micrographs of BTV-8**





# 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

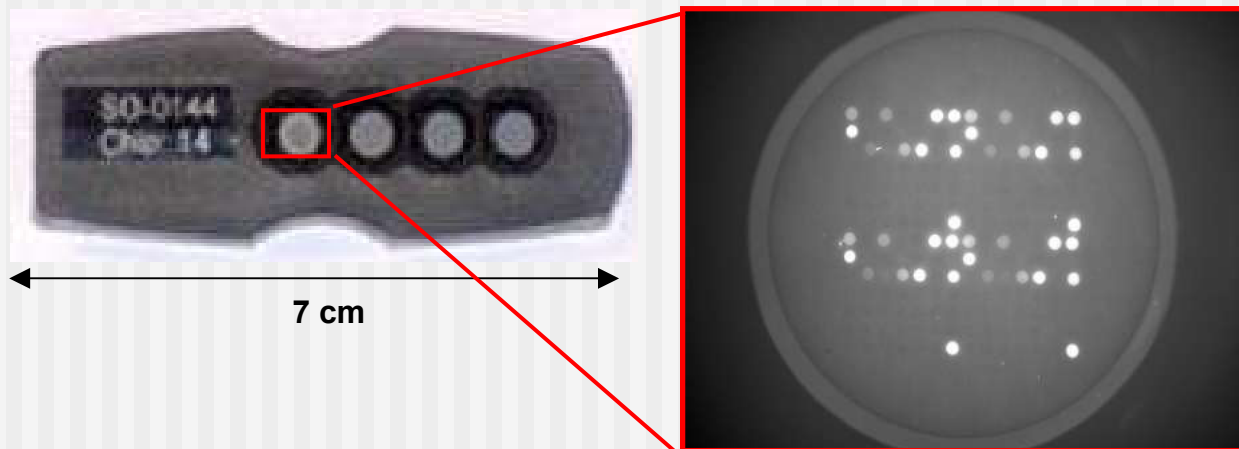
**PHILOSOPHY** : 1 suspect sample, 1 test for all agents ...

**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



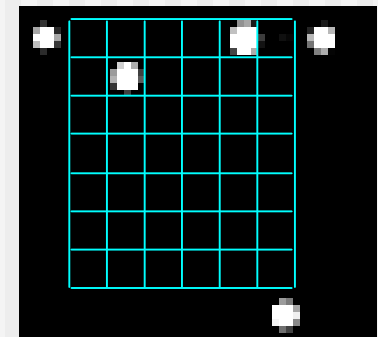
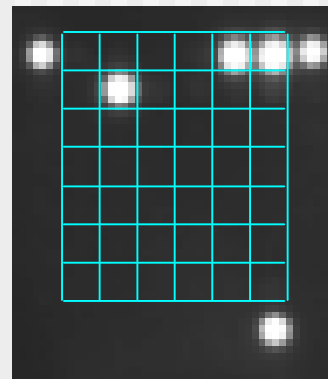
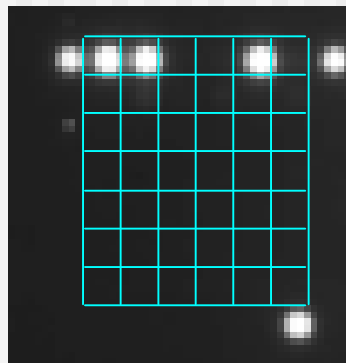
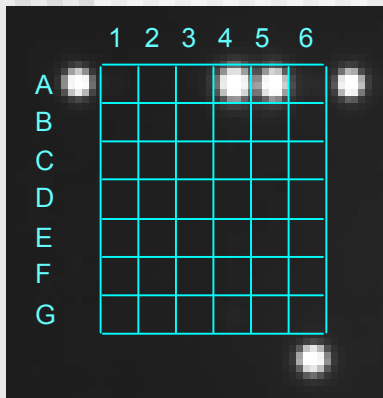




# First results

DNA source	conc. (ng) assay	PCR	plcR		pla		23S		narK18		IS1111		fopA		Vir1		Vir2		Vir4		Vir5	
		contr ol	CAP	313T	A3	A4	A5	A6	B0	B1	B2	B3	B4	B5	C0	C1	C2	C3	C4			
Y pestis NCTC8789 <sup>1</sup>	16	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
B anthracis EQAE2 <sup>1</sup>	10	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
B mallei NCTC10229 <sup>1</sup>	14	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	
B pseudomallei NCTC1688 <sup>1</sup>	14	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	
Coxiella (PCR IS + SS DNA) <sup>1</sup>	1	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	
Francisella tularensis EQAE9 <sup>1</sup>	3.6	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	
Brucella <sup>1</sup>	28	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	
Plasmid-cloned SPPV <sup>2</sup>	2	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
Plasmid-cloned GTPV <sup>2</sup>	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	
Plasmid-cloned SGPV <sup>2</sup>	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	

<sup>1</sup>Qiagen prep's (QG DNA Easy kit)  
<sup>2</sup>Plasmid 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

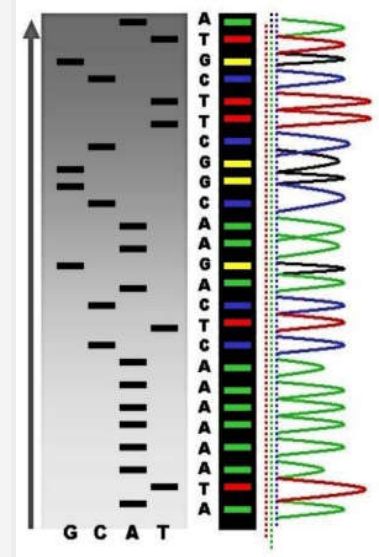
- LDR lasts min. 10 hours (improvable)
- Limited array capacity ( $\pm 100$  spots)
- Limited sensitivity





# Random DNA sequencing

- **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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- Conclusions





# 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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- 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







# 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.





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