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

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CODA-CERVA

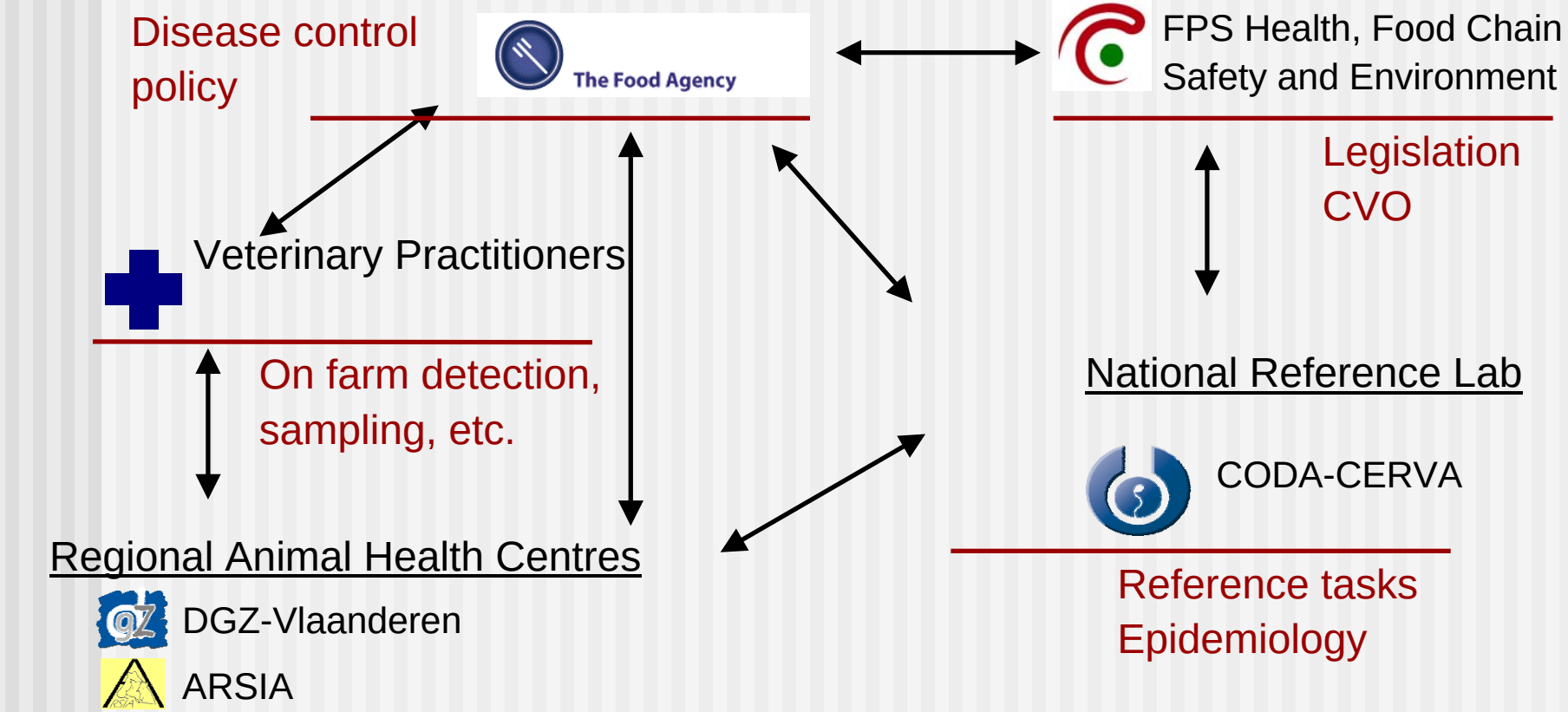
Contents

- 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



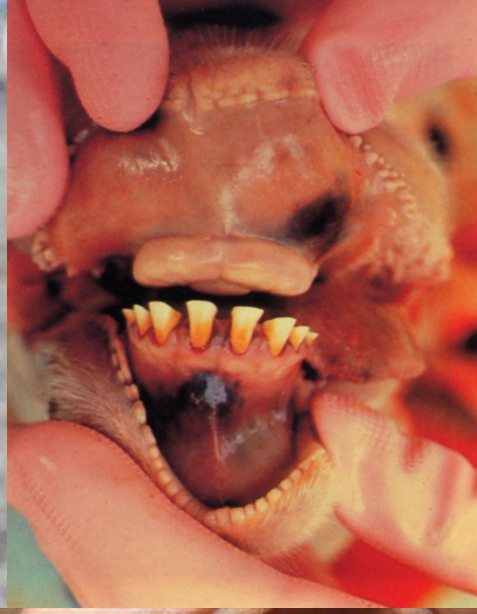
Most recent example: Belgium

- 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.

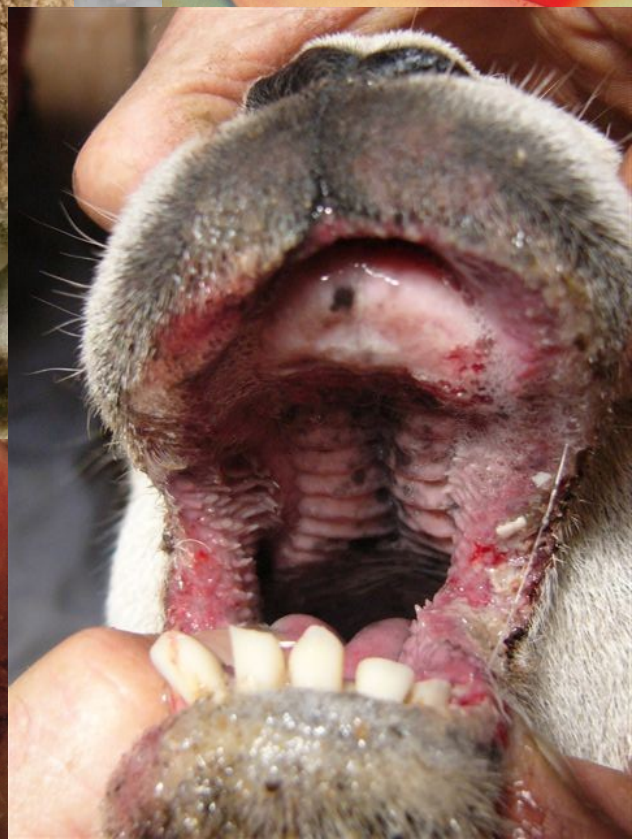
… and in The Netherlands

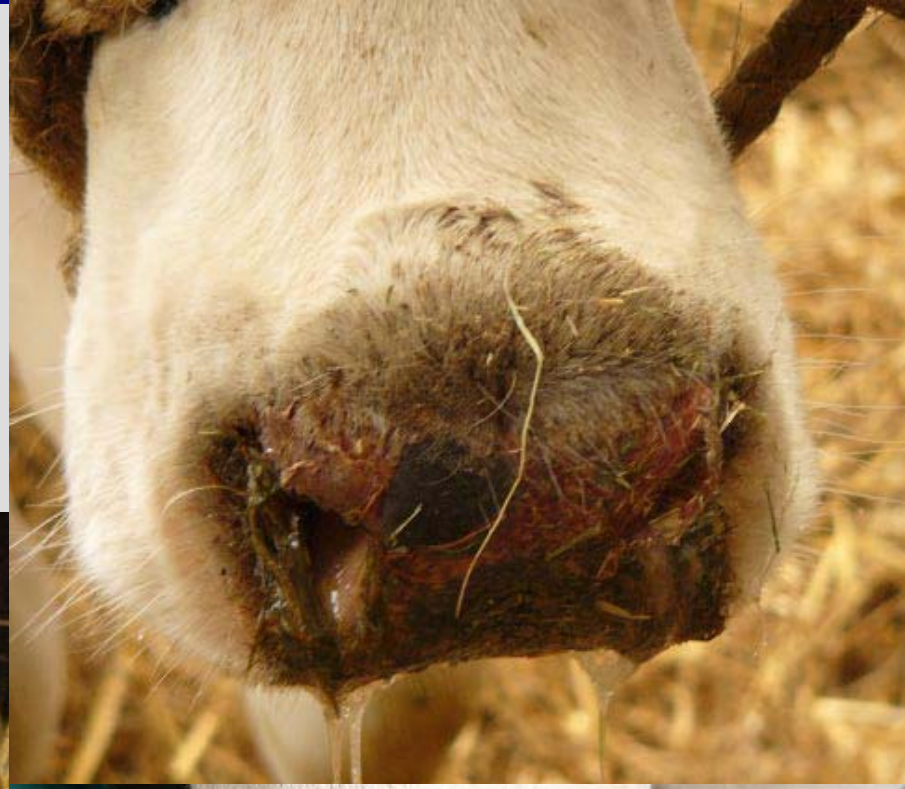
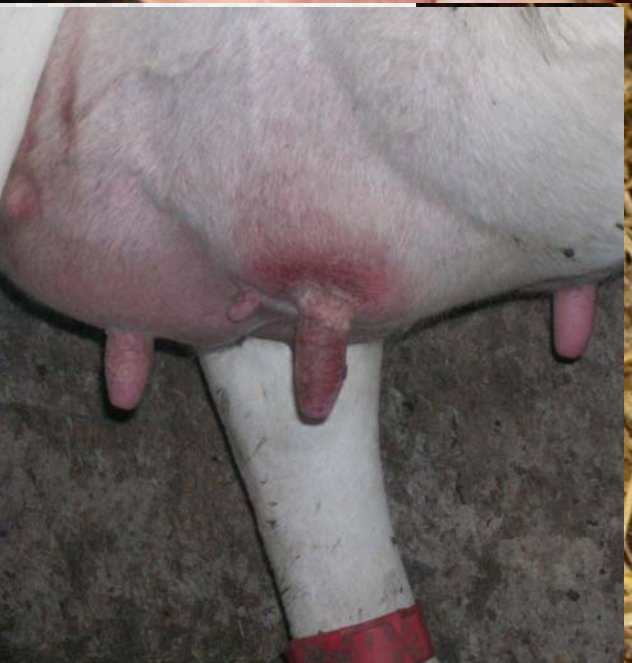
- Since the beginning of August, a few veterinary practitioners in southern part of The Netherlands observed mandibular oedema and oedema of the lips in a few sheep in sheep flocks and consulted the helpdesk of the Animal Health Service Ltd.
- Further consultations of the helpdesk indicated that these 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

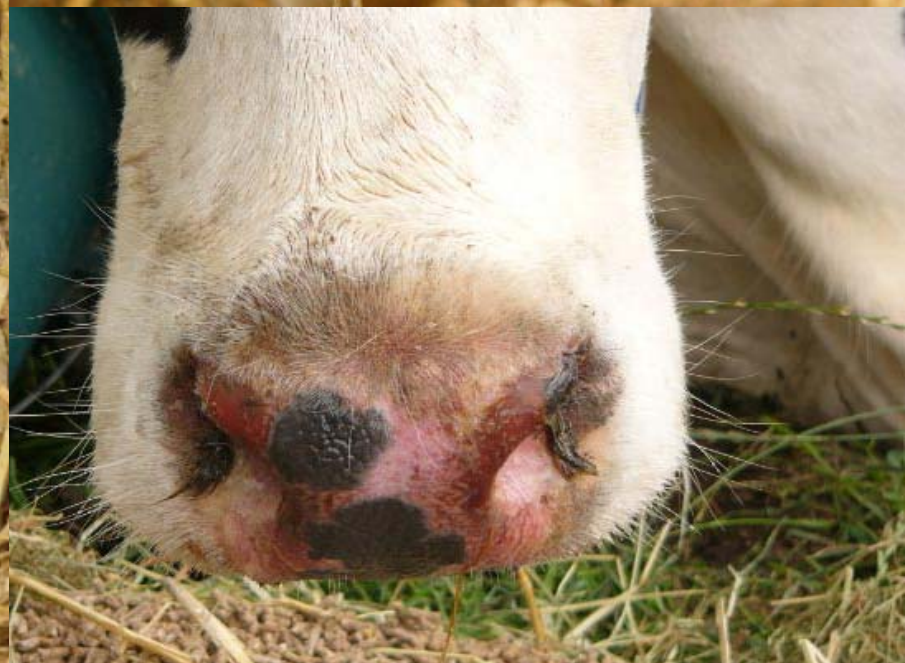










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What are the challenges?

- 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.

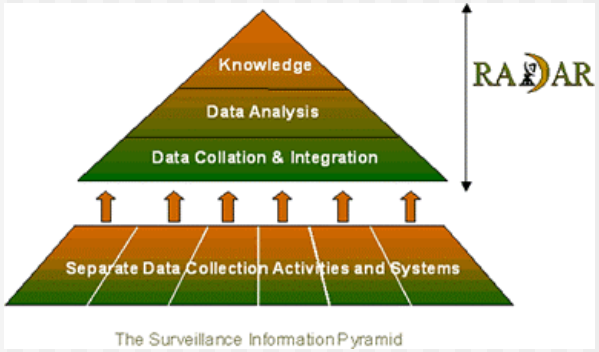
Rapid knowledge of the epidemiological evolution

- 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 Surveillance Information Pyramid

- 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

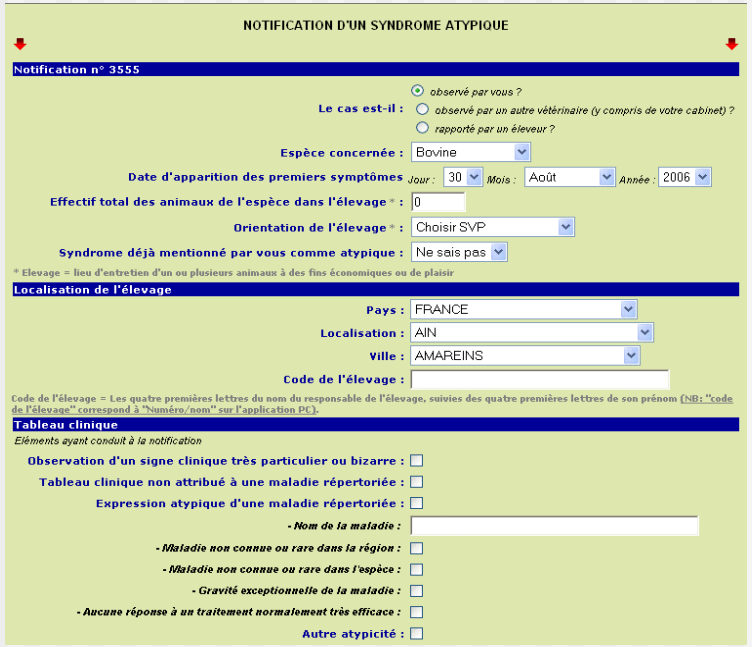
RADAR facilitates

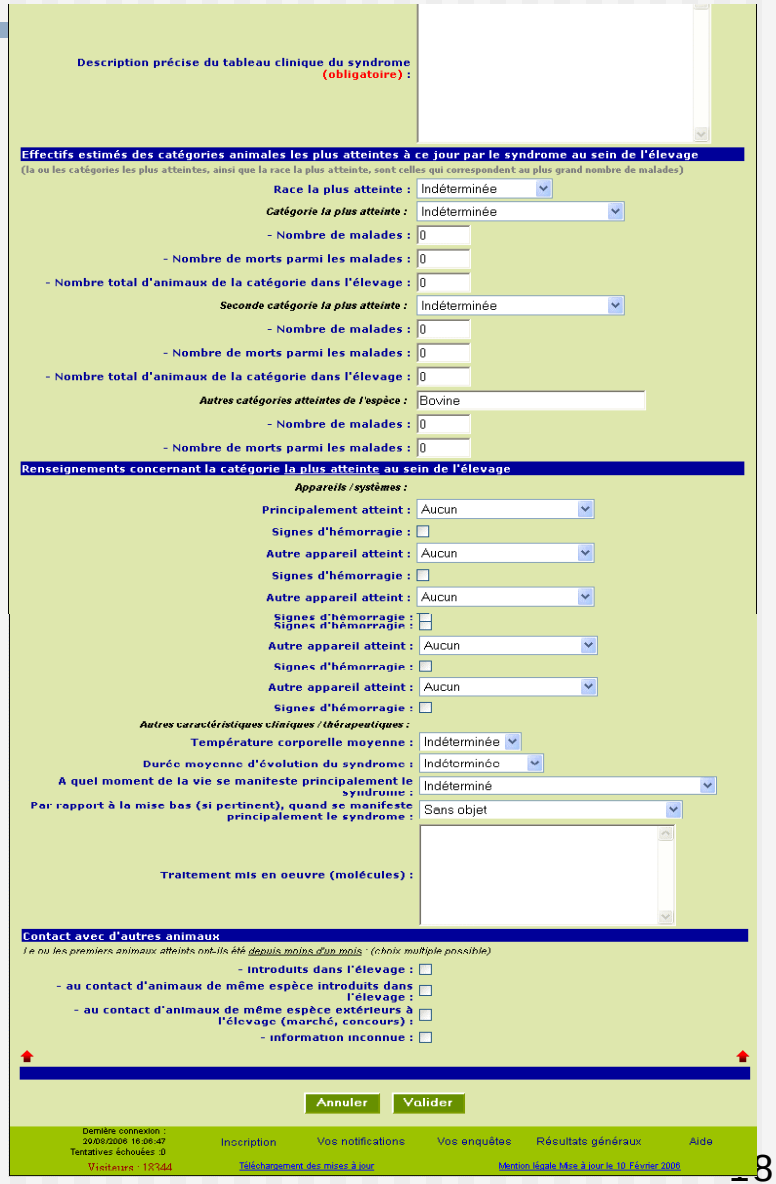
- Earlier detection / intervention of new & emerging diseases
- Improved chance of detecting links between human & animal disease
- More effective targeting of resources for surveillance & control
- Policy making will be based on improved evidence of disease risk and impact
- Better informed stakeholders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

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





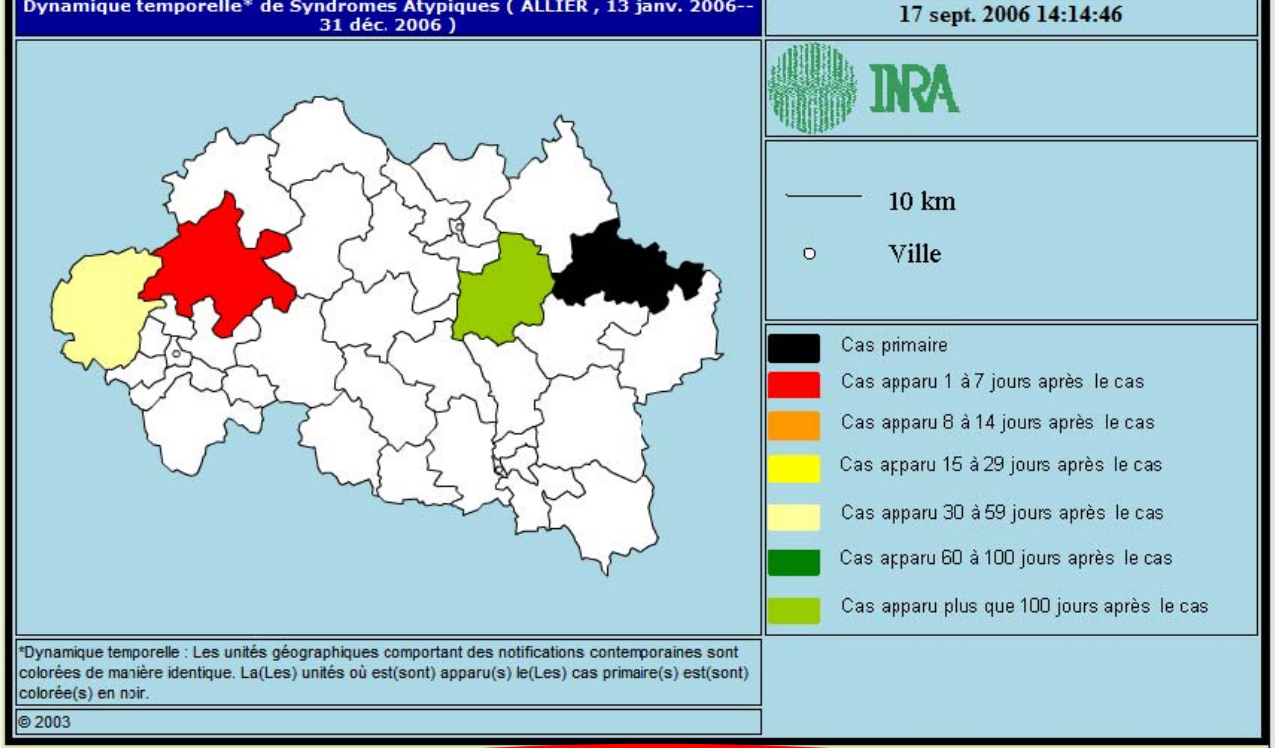
- Initial notification and automatic follow-up
- Compromise between precision - ease of use
- 3 geographic levels (country, department, region)
- Automatic alert of experts after classification

Info and real-time statistics

Dynamic geographical analysis

INRA émergences.

CARTOGRAPHIE



Distance entre les 6 premiers cas		
	Distance temporelle	Distance spatiale
Cas primaire : DOMPIERRE-SUR-BESBRE 0307 -- cas 2 : HERISSON 0312	5 jours	79 km
Cas 2 : HERISSON 0312 -- cas 3 : HURIEL 0313	51 jours	23 km
Cas 3 : HURIEL 0313 -- cas 4 : HURIEL 0313	79 jours	0 km
Cas 4 : HURIEL 0313 -- cas 5 : HURIEL 0313	37 jours	0 km
Cas 5 : HURIEL 0313 -- cas 6 : NEUILLY-LE-REAL 0325	61 jours	78 km

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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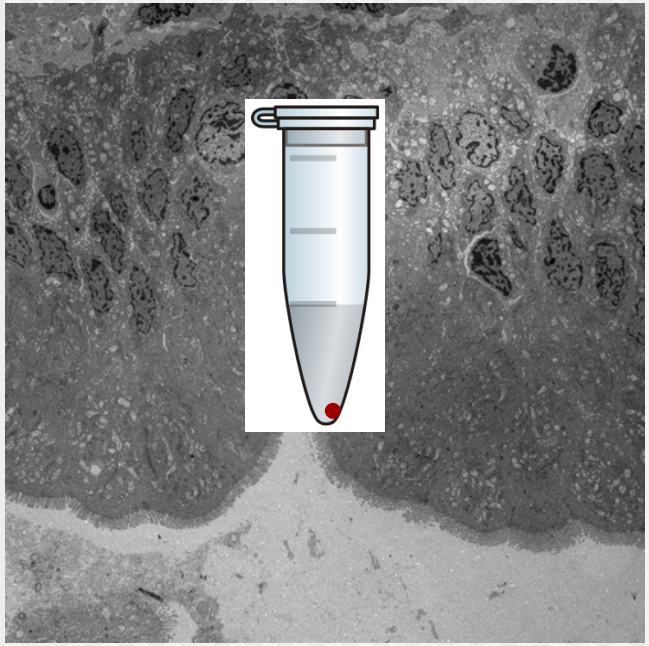
Global analyses

“All catching techniques”

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

Resolving power of EM

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)
■Non-specific ■Non-directed ■Open-view ■No a priori choice of pathogen	■Specific test fail ■Specific tests are unavailable ■Pathogens are new, rare, changed ■Pathology / question is complex ■Orientation required
■ (Can be) rapid	■The situation is urgent ■People are impatient, curious
■Independent confirmation ■Morphology-based confirmation	■To avoid / end discussions ■“Pictures don’t lie” ■“One image is worth a thousand words” ■To help people better

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

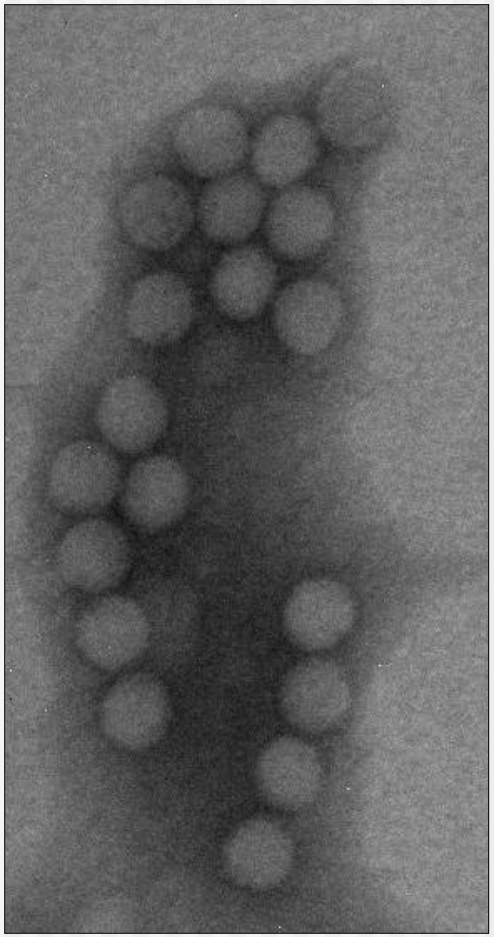
- Several outbreaks of viral zoonoses
  (SARS, monkey pox USA, Avian influenza, Hendra virus, West Nile disease, ...)
- Each time, EM was important:
  - As a first-line test, for orientating diagnosis
    (no need of specific reagents)
  - For independent confirmation of other test results
  - To characterise the morphology and morphogenisis of the virus
- EM will certainly be used in a next crisis.

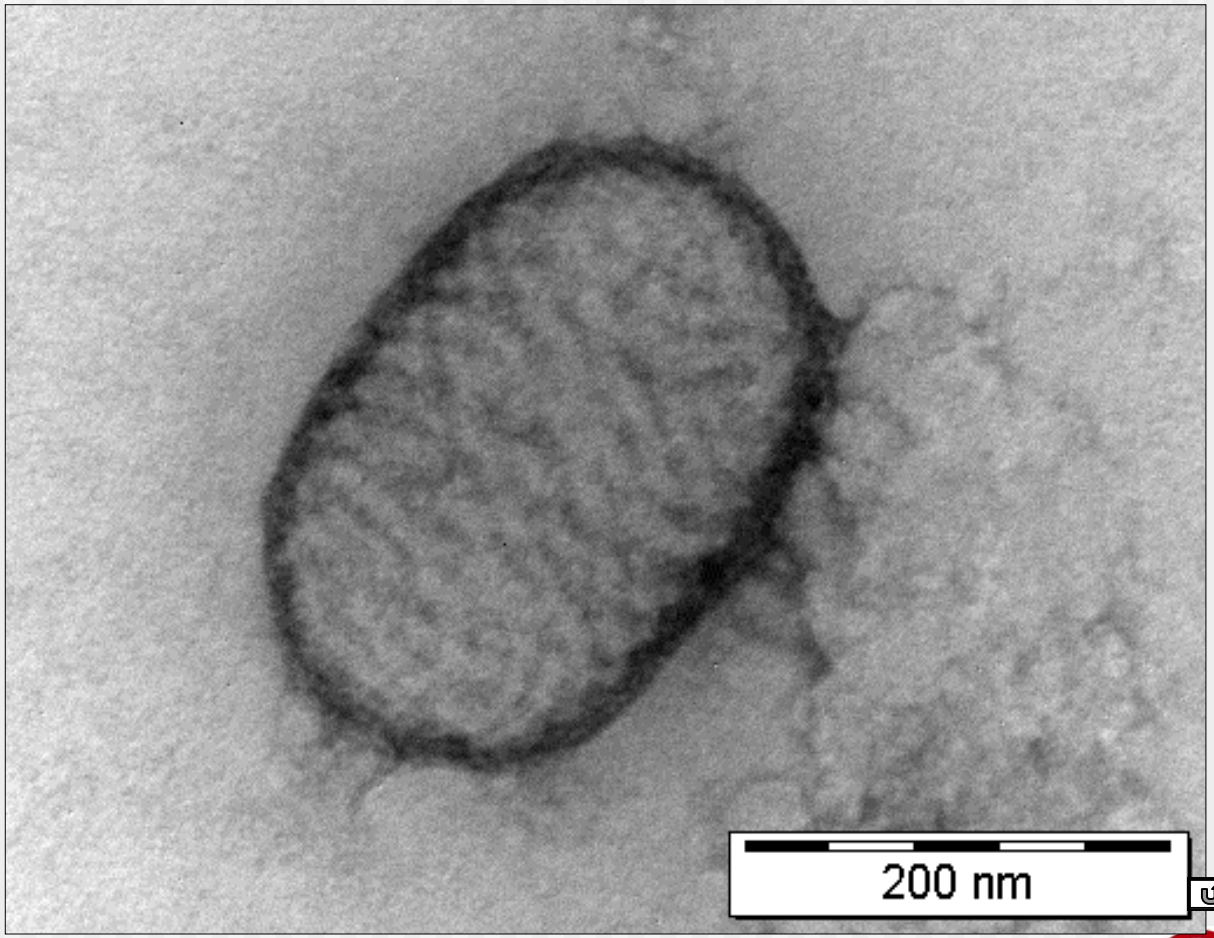
➔For specific virus families containing candidate new, emerging and rare virus, **insufficient expertise** of morphology and morphogenesis is available.

Objectives

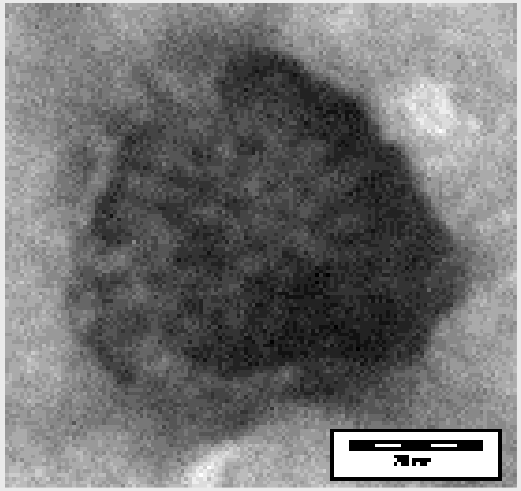
- (Maintain and develop expertise – the only unit in Belgium)
- Pro-active characterisation of candidate emerging diseases.
  - Using standard and advanced EM techniques
    - Negative staining
    - Ultra-thin sectioning
    - Immuno-EM Immunogold NDV
    - Cryo-EM
    - Electron tomography
  - Well-chosen models of specific virus families

Urgent differential diagnosis FMDV - Parapoxvirus

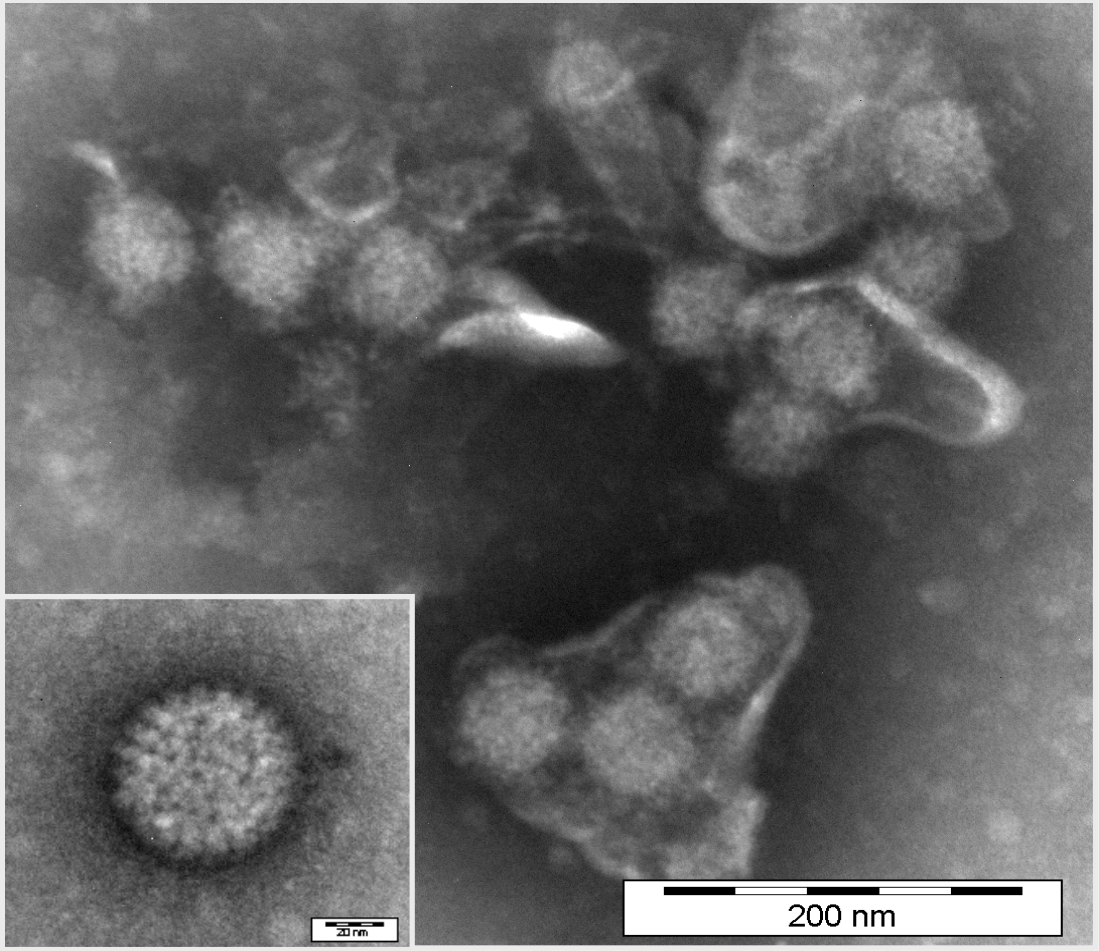




EM confirmation of BTV-8 in Belgium



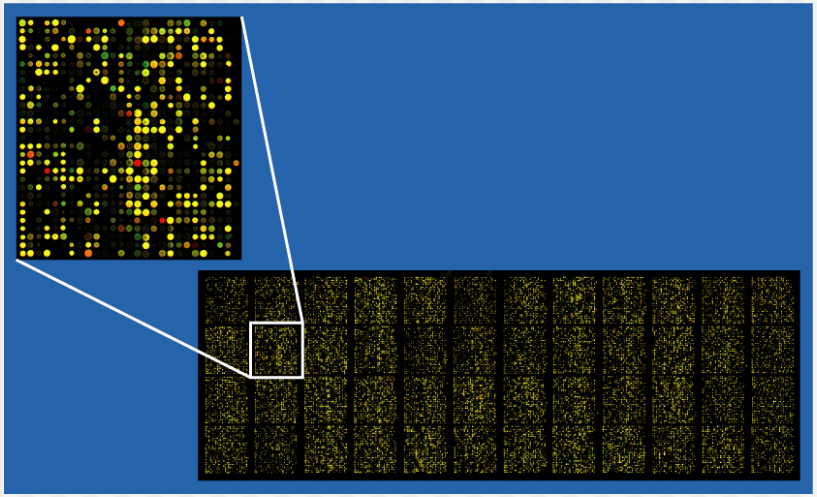
First BTV-8 virion observed in Europe (bluetongue crisis 2006)



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

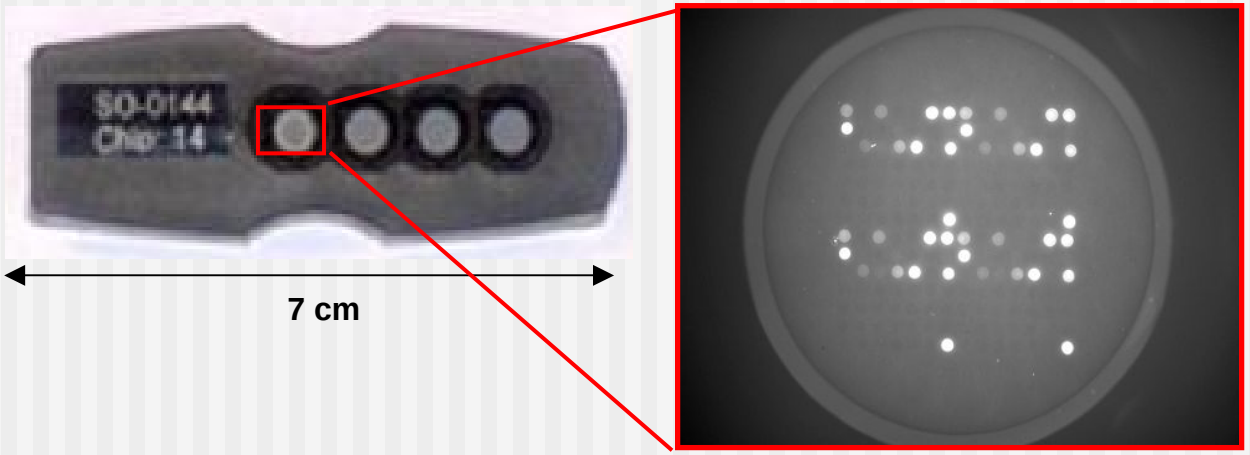
PHILOSOPHY : 1 suspect sample, 1 test for all agents …

Array Format : PAMGENE™

- Fluorescence detection, liquids are flushed through
- Single tube technology (multiplexed hybridization reactions)

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

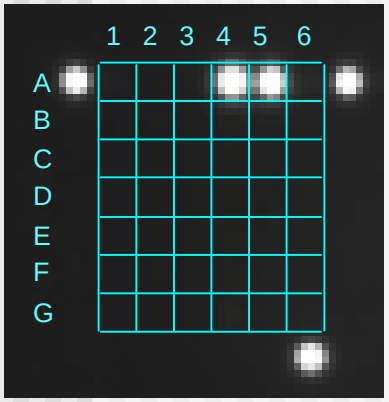
Initial probe design for 7 BSL-3 bacteria and 4 animal viruses

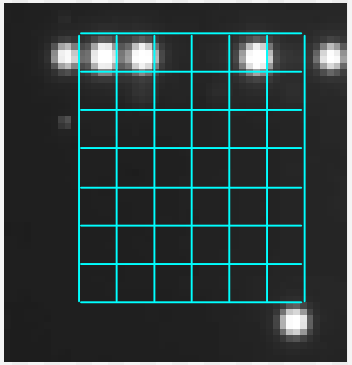


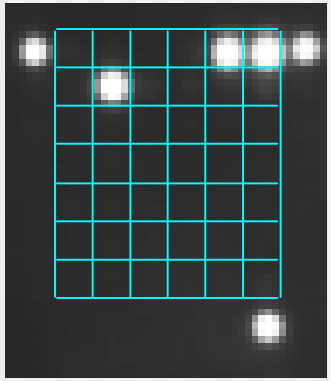
First results

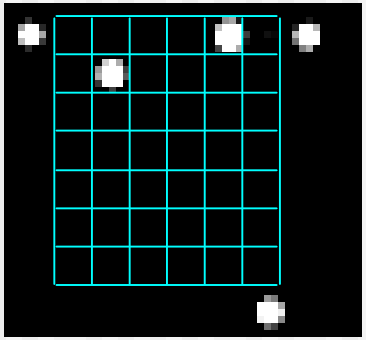
		PCR control	CAP	plcR 313T		pla	23S rDNA	narK18			sctV	IS1111 A	fopA	IS711	Vir1	Vir2		Vir4	Vir5
DNA source	conc. (ng) assay	A0	A1	A2	A3	A4	A5	A6	B0	B1	B2	B3	B4	B5	C0	C1	C2	C3	C4
Y pestis NCTC8789[1]	16		-	-		+	+	-			-	-	-	-	-	-		-	-
B anthracis EQAE2[1]	10		+	+		-	+	-			-	-	-	-	-	-		-	-
B mallei NCTC10229[1]	14		-	-		-	+	+			+	-	-	-	-	-		-	-
B pseudomallei NCTC1688[1]	14		-	-		-	+	-			+	-	-	-	-	-		-	-
Coxiella (PCR IS + SS DNA)[1]	1		-	-		-	-	-			-	+	-	-	-	-		-	-
Francisella tularensis EQAE9[1]	3.6		-	-		-	+	-			-	-	+	-	-	-		-	-
Brucella[1]	28		-	-		-	+	-			-	-	-	+	-	-		-	-
Plasmid-cloned SPPV[2]	2		-	-		-	-	-			-	-	-	-	+	-		-	-
Plasmid-cloned GTPV[2]	2		-	-		-	-	-			-	-	-	-	-	+		+	-
Plasmid-cloned SGPV[2]	2		-	-		-	-	-			-	-	-	-	-	-		-	+

[1]Qiagen prep's (QG DNA Easy kit)
[2]Plasmid miniprep + restrictie + EtOH precipitation







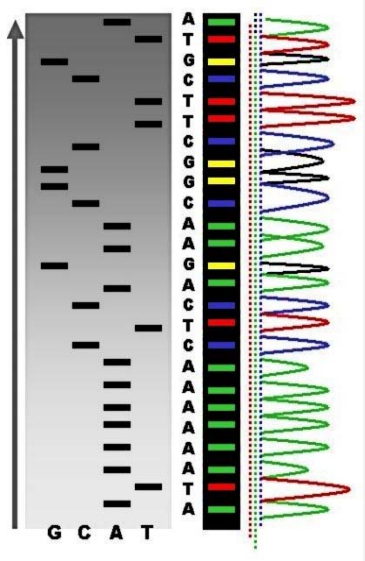


Evaluation of PAMGENE format

- **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 (± 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 technologes 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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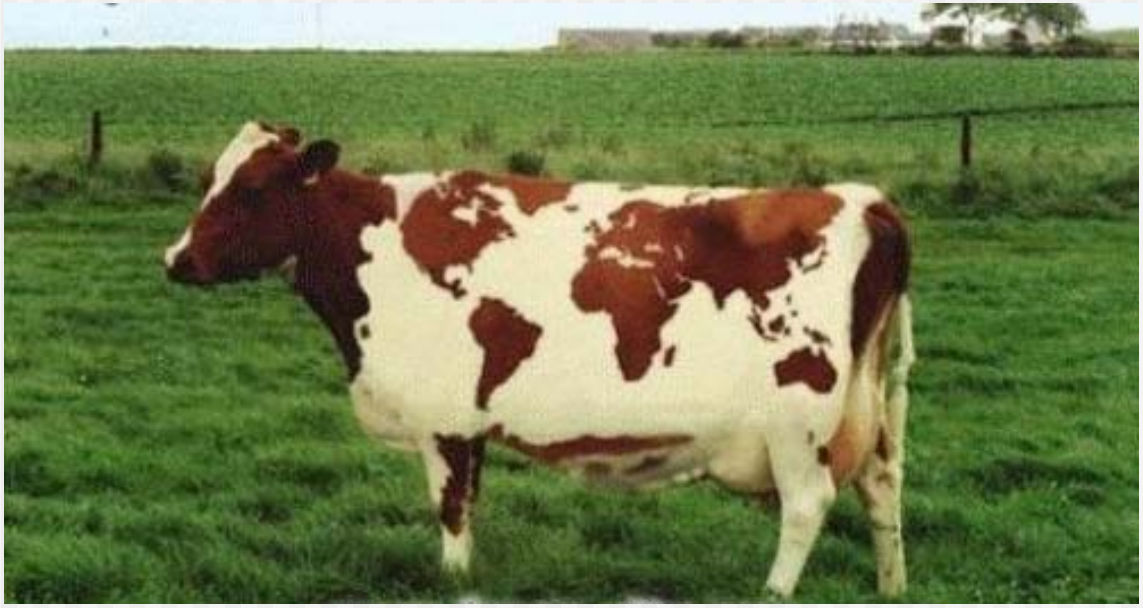
To face successfully the next emerging disease

- 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

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  - MOSS: M. Dispas, C. Herr
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  - DNA sequencing: S. van Borm
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