FMD, warning system and diagnostic methods: a French expertise

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FMD/SVD/VS NRL
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ANSES Laboratories

- Fougères: Laboratoire de Fougères
  - Agence nationale du médicament vétérinaire
  - Antibiotiques et résidus de colorants

- Dozulé: Laboratoire de pathologie équine
  - Maladies équines (autres que peste équine) - labo. associé

- Boulogne-sur-Mer: Laboratoire des produits de la pêche

- Nancy: Laboratoire de la rage et de la faune sauvage
  - Laboratoire d'hydrologie

- Maisons-Alfort: Laboratoire de la santé animale
  - Tuberculose bovine
  - Paratuberculose
  - Brucellose
  - Maladies touchant autres que peste équine - labo. associé

- Angers: Laboratoire de la santé des végétaux
  - Rhinotrachéite de la dinde
  - Bourse infectieuse
  - Maladie d'Aujeszky

- Niort: Laboratoire de Niort
  - Viroses bovines
  - Arbovirus et encéphalites

- Lyon: Laboratoire de Lyon
  - Pépinière bovine contagieuse

- Nice: Laboratoire de Sophia-Antipolis
  - Maladies des abeilles
  - Santé des abeilles

- Plofragan-Plozané: Laboratoire de Plofragan-Plozané
  - Rhinotraceyté de la dinde
  - Bourse infectieuse
  - Maladie d'Aujeszky
BioPic TEAM

NRL: FMD – SVD - VSD

1 - Ensure the diagnosis (primary mission and priority)
   - Emergency (24h/24h)
   - Outbreaks
   - Surveillance post-outbreaks (manage regional lab Network)
   - International trade

2 - Provide expertise to policy makers

3 - Develop research projects:
   - Improvement of diagnosis
   - Development of new vaccine approaches
   - Study virus/host interaction: Mechanism of virus persistence/virulence
   - International cooperation projects: Morocco, Tunisia, Algeria, Benin…
Foot-and-Mouth Disease (FMD)

Old disease but actual threat...

1514
Italy
Disease description

1898
Identification of the agent

2011

1916 Cattle Diseases, USDA

UK 2001

JAMES CHAPMAN and KIRSTY WALKER
Foot-and-Mouth Disease (FMD)

- Highly contagious viral infection of cloven-hoofed domestic and wild animal
- Characterized by vesicles, with subsequent erosions in the mouth and also on the muzzle, feet, or teats.
- High morbidity, low mortality
- Carriers animals

(Virus present in oropharynx more than 28 days after infection)
Family: Picornaviridae
Genus: Aphtovirus

- Non-enveloped Icosahedral capsid
- Genome ssRNA+, 8.2 kb
- 7 serotypes (O, A, Asia 1, C, SAT 1-3)
- Quasi species

- Spread rapidly and easily
- Resistant in environment
Regional FMDV Pools

Pool positions are approximate and colours indicate that there are three principal pools, two of which can be subdivided into overlapping areas.

Paton D J et al. Phil. Trans. R. Soc. B 2009
Spread of FMDV (EU)

**UK 2001:**
- 2030 outbreaks
- 6 million animals destroyed
- £3.1 billion (Agriculture)
- £2.7–3.2 billion (tourism)

**France, 2001:**
- 2 outbreaks
- 58,000 animals destroyed
- 1 week embargo: 40 millions €
Japan, April-August 2010 (Miyazaki)

-Serotype O-

292 Outbreaks
211,608 animals destroyed
3.14 billion USD
South Korea, Beginning: Late Nov 2010

-Serotype O-

175 outbreaks
3.3 millions Animal Destroyed
1.8 billion USD
FMD knock on the door of Europe again...

Bulgaria (near the border with Turkey)

Beginning: 04 January 2011
First case in 12 years (FMD case in a shot wild boar) - Serotype O

12 Outbreaks
1372 Animals Destroyed

! Free countries! are vulnerable to FMD
Protecting animals against FMDV is based on a dual system:

- Prevention measures based on monitoring and protection of farms,
- Fight against FMD with the deployment of emergency plans in an outbreak situation.

Two Objectives:

- Avoid the introduction of the disease,
- Immediately detect the disease in order to contain and eradicate it as quickly as possible.
Control of Foot-and-Mouth Disease

Control can be reached by implementing of measures to:

- prevent the disease from entering the territory,
- detect the disease as early as possible (warning system),
- manage the first suspected outbreaks,
- manage the confirmed outbreaks,
- eradicate the disease,
- justify the end of the outbreaks.

Their application involves different actors whose:

- actions must be collective and coordinated,
- competence, efficiency and responsiveness must be strengthened and maintained regularly.
Diagnostic Laboratory
Role of Diagnostic laboratory

- Confirms clinical diagnosis
- Supports but does not replace the need for accurate clinical diagnosis
- The quality of the laboratory diagnosis depends on the selection and quality of the samples
- Requires full epidemiological information on samples submitted for the choice of tests to perform and rational interpretation of the results
FMDV Diagnosis

Uninfected → FMDV infected → Recovered (or vaccinated)

Samples of lesions or blood

Blood samples

Virus or viral components can be detected

Virological Diagnosis

Serological Diagnosis

Live Virus → Viral Proteins → Viral Nucleic Acid → Anti-FMDV antibodies
SEROLOGICAL DIAGNOSIS OF FMD
Replication cycle of the virus
Virus replication in the animal produces structural (capsid) proteins, which form virus particles, and non-structural proteins, with enzymatic and regulatory functions.

Infected animals produce antibody to both structural (capsid) proteins and non-structural proteins.

The animal receives predominantly structural proteins in the form of purified virus particles and small amounts of non-structural protein contaminants.

Vaccinated animals produce antibody primarily to structural (capsid) proteins and respond only weakly to contaminating non-structural proteins.

Can discriminate between infected and vaccinated animals on basis of differential antibody response to structural and non-structural proteins.
Serological Methods

Antibodies against NSP

Kit Priocheck NSP

SVANOVIR® FMDV 3ABC-Ab ELISA

Anti-bovine-IgG1 conjugate
Ab in samples
3ABC Ag

For any Serotype

Antibodies against SP

SPCE

Kit Priocheck Type O

Virus Neutralization Test

LPBE

Serotype specific
Application

1- to certify individual animals prior to import or export
2- to confirm suspected cases of FMD
3- to confirm freedom following outbreak
4- to differentiate infected from vaccinated animals
5- to demonstrate the efficacy of vaccination
VIROLOGICAL DIAGNOSIS OF FMD
FMD virus detection

Where to find the virus?

Blood

Nasal/Buccal swabs

Oesophagopharyngeal (probang) fluid

Milk

Vesicular fluid
And epithelial tissue

Also in Heart and other organs of fatal cases
Methods for FMDV detection and typing

**Antigen detection**

- Virus isolation on cell culture (detection)
  ![Virus isolation on cell culture](image1)
- Indirect sandwich ELISA (detection and typing)
  ![Indirect sandwich ELISA](image2)
- Lateral flow device test (detection)
  ![Lateral flow device test](image3)
- Complement fixation test (detection and typing)
  ![Complement fixation test](image4)

**Genome detection**

- Real time RT-PCR (detection, typing)
  ![Real time RT-PCR](image5)
- Classical RT-PCR (detection, typing and molecular epidemiology)
  ![Classical RT-PCR](image6)
Application

In case of suspicion ➔ rapidly confirm or not FMDV infection for decision making

In case of outbreak ➔ rapidly detect and type the FMDV to implement adequate measures

During an outbreak ➔ rapidly detect any new introduction and change of the circulating strain
warning system and diagnostic methods: French expertise

NRL (Maisons-Alfort)
High security laboratory facilities
Epidemiologists
Virologist
Laboratory Technicians

Veterinary Laboratories
5 of 83 are accredited for the serological diagnosis of FMD (01, 22, 29, 72, 81)

High security laboratory facilities
Localisation
Missions of NRL

- Provide the Ministry of Agriculture, expertise and technical support,
- **Perform the first-line analysis of suspected cases of FMD,**
- Develop, optimize and validate analytical methods and participate in their normalization,
- Evaluate diagnostic tests,
- Collaborate with the World Reference Laboratory and other European or International Laboratories,
- Provide scientific and epidemiological monitoring,
- Conduct research (ex. participation to European projects).
- Animate network of accredited laboratories by: organization of training, transfer of diagnostic methods and organization of proficiency testing,
- **If necessary, confirm the results of tests performed by a laboratory of the network,**
Role of Veterinary Laboratories

- Participate in the evaluation and validation of serological methods.
- Provide support for serological investigations (ELISA) and virus detection (RT-PCR) during outbreaks.
- Provide support for serological surveillance (ELISA) after slaughter or vaccination.

Peace evaluation and validation

OUTBREAKS

Serological surveillance (ELISA)
Genome detection (RT-PCR)

Monitoring

Serological surveillance (ELISA)

Peace
warning system in France

Veterinarian

Farmer

DDPP

DGAL

NRL Epidemiologist on guard

NRL Virologist on guard

Preparation of diagnosis 24h/24h

Staff NRL

Results

Phone 24h/24h

E. Mail

Information and Photos

shipment of samples
Scenario: Sunday 06/02/2011 at 6pm, VS in South of France call for suspicion of FMDV infection in farm of 100 bovines. Recently, the farm introduced two bovines coming from west Africa. Epidemiologist of the NRL confirm the suspicion and call the virologist at 8pm. Virologist call the VS to prepare shipment of samples. VS collected 3 samples of epithelium in transport medium and sent samples by route. Samples will arrive 07/02/2011 around 8 am.

Samples arrived to lab at 9 am and received by lab staff (3 technicians).
FMDV detection and typing in an emergency situation

Déroulement des analyses

Fiche de réception en cas de suspicion FA

Nom du Virologiste de garde : Bakkali Kassimi Labib

Notification d’alerte (Nom de la personne déclenchant l’alerte, la date, et l’heure) :

Gina Zanela, 06/02/11, 19h55

Coordonnées de la personne responsable de l’envoi des prélèvements :

DDPP 66

Date et Heure du contact téléphonique : 06/02/11 à 20h00

Echange d’information entre virologiste de garde et la DDPP :

- Elevage contenant 100 bovin.
- Vu aphtes sur des bovins qui boitaient, et aphtes sur la bouche.
- Prélèvements envoyés dans la soirée en voiture du sud de la France
- Animaux importés du Bénin marché Guéma PARAKOU (Importation 26/07/10)

Arrivée des prélèvements :

<table>
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<tr>
<th>Personnels</th>
<th>Date et heure</th>
<th>Nom et Visa</th>
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<tbody>
<tr>
<td>Société de livraison : Labo FA</td>
<td>07/02/11 à 9h00</td>
<td>Labo FA</td>
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<tr>
<td>de l’accueil : Labo FA</td>
<td>07/02/11 à 9h00</td>
<td>Labo FA</td>
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<tr>
<td>du laboratoire : Kamila Gorna</td>
<td>07/02/11 à 9h00</td>
<td>KG</td>
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Conditionnement des prélèvements :

<table>
<thead>
<tr>
<th>État du colis</th>
<th>Conformité</th>
<th>Observations</th>
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<tbody>
<tr>
<td>Aspect général</td>
<td>Oui ☒ Non ☐</td>
<td></td>
</tr>
<tr>
<td>Triple emballage</td>
<td>Oui ☒ Non ☐</td>
<td></td>
</tr>
<tr>
<td>Récipient</td>
<td>Oui ☒ Non ☐</td>
<td>Tubes en plastique mal étiquetés</td>
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</table>

Numéro de dossier interne (Numéro-Année-N° département) : 01-11-Béni

Suspicion and samples registration
## Lambeaux d’épithélium/Date et heure de début de traitement : 9H15

<table>
<thead>
<tr>
<th>Lèvement</th>
<th>Conditionnement/poids</th>
<th>Poids utilisé</th>
<th>Volume de dilution</th>
<th>Surnageant après centrifugation</th>
<th>Surnageant utilisé</th>
<th>Surnageant stocké</th>
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<tbody>
<tr>
<td></td>
<td>Sec</td>
<td>pH : 7,5</td>
<td>0,77g</td>
<td>1/5 + 3,08 ml</td>
<td>NA</td>
<td>2650µl</td>
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<tr>
<td></td>
<td>Milieu de transport</td>
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<td></td>
<td></td>
<td>1350µl</td>
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<td></td>
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<tr>
<td></td>
<td>NA</td>
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<td></td>
<td>430µl</td>
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</table>

Heure de fin de traitement : 10h25

Date et Heure de fin de traitement : 07/02/11 à 10h25
FMDV detection and typing in an emergency situation

Analysis

Samples (10h25)

- LFD (Svanodip) St 10h25
- Ag ELISA St 10h45
- rtRT-PCR St 10h45

Virus isolation St 11h

10h35 S1 (+)
10h55 S2 doubtful and S3 (-)
14h30 S1 (+) type O, S2 and S3 (-)
15h30 the 3 samples (+)

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<th>3D</th>
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<tr>
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<td>25,28</td>
<td>29,02</td>
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<td>S2</td>
<td>35,44</td>
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<td>S3</td>
<td>34,77</td>
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FMDV detection and typing in an emergency situation

### Analysis

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<th>6 PM</th>
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<td>IBR</td>
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</tr>
<tr>
<td>S1</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>S2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

End of diagnosis 6PM

confirmation and typing in ~6 h
Conclusion

✓ FMD old disease but is still a great concern for animal health and a threat to many countries.

✓ The success of the control of FMD depends of:
  ➤ The existence of a network of actors whose actions should be collective and coordinated,
  ➤ Strengthening and maintaining the network regularly
  ➤ An effective Warning System and emergency plan.
  ➤ A very good vaccine (effective and DIVA)

✓ One key point in the control of FMD is the early detection and typing of FMDV:
  ➤ Network of laboratories
  ➤ Adequate resources and staff qualified and well trained.
  ➤ Full epidemiological information and good quality of samples
  ➤ Sensitive and specific diagnostic methods
Can we eradicate FMD in the world one day?

Thank you for your attention.