

FMD, warning system and diagnostic methods: a French expertise

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FMD/SVD/VS NRL

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www.anses.fr



French Agency for Food, Environmental
and Occupational Health & Safety



-  ANSES
-  **Food** >>
-  Animal health and nutrition
-  Environmental health
-  Work environment
-  Plants



News Opinions, reports

Publications

6 October 2011
ANSES celebrates science! (in French)

Health warning - 5 October 20
**Dot not use veterinary medicines to
eliminate lice on children**

Agenda (French only)

18 November 2011
**ANSES Day for Animal Antimicrobial
Resistance (in French)**

> See all events

Focus on



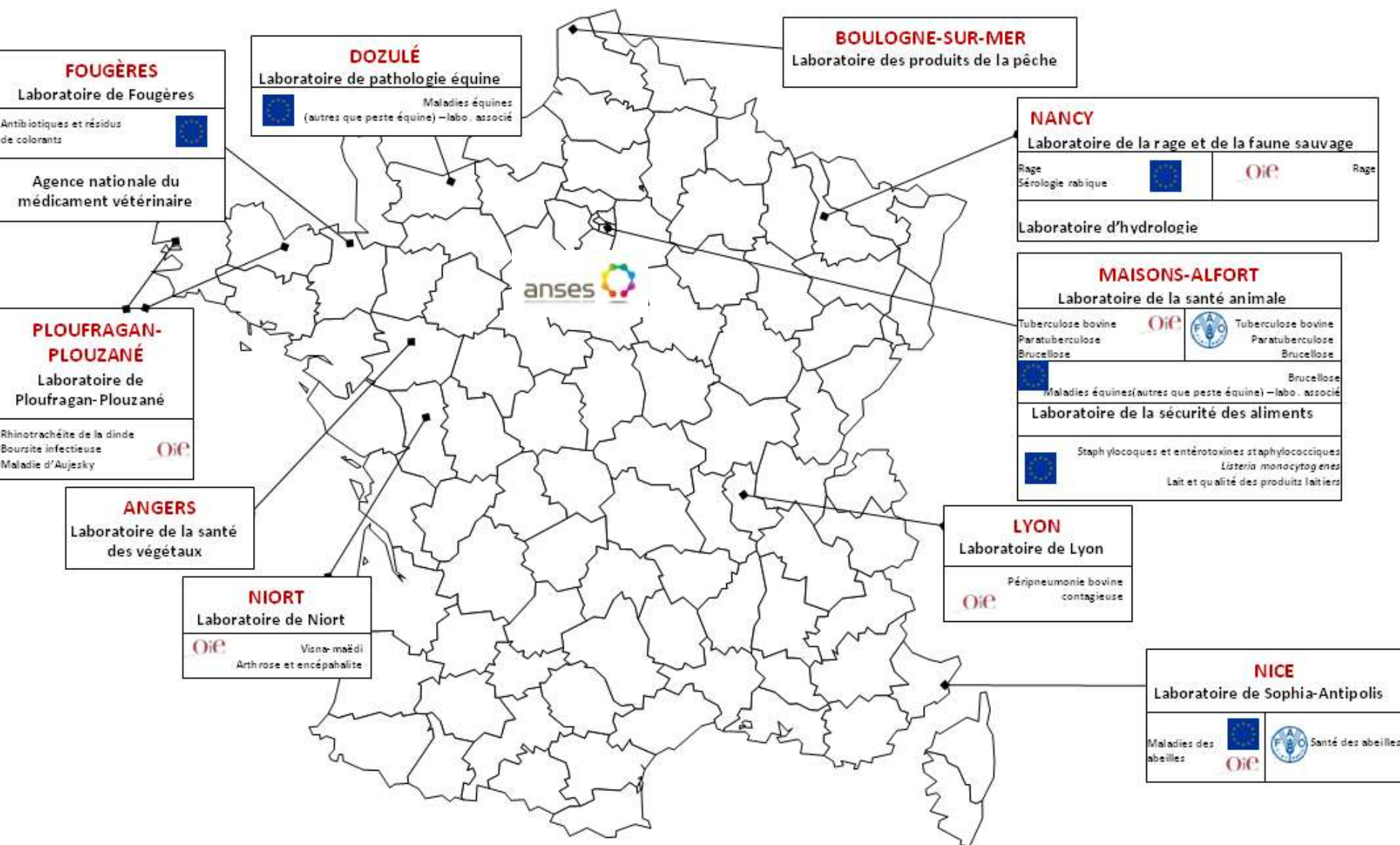
Press Room

Research program

Laboratories

Calls for expertise

ANSES Laboratories



JOINT RESEARCH UNIT OF VIROLOGY 1161



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BioPic TEAM

NRL : FMD – SVD - VSD

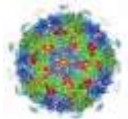
1 - Ensure the diagnosis (primary mission and priority)

- ✓Emergency (24h/24h)
- ✓Outbreaks
- ✓Surveillance post-outbreaks (manage regional lab Network)
- ✓International trade

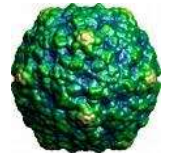
2 - Provide expertise to policy makers

3 - Develop research projects:

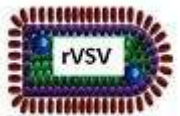
- ✓Improvement of diagnosis
- ✓Development of new vaccine approaches
- ✓Study virus/host interaction: Mechanism of virus persistence/virulence
- ✓International cooperation projects: Morocco, Tunisia, Algeria, Benin...



FMDV



SVDV



VSDV



FMD-Disconvac



Rapidia



AniBioThreat



EMIDA
ERA-NET

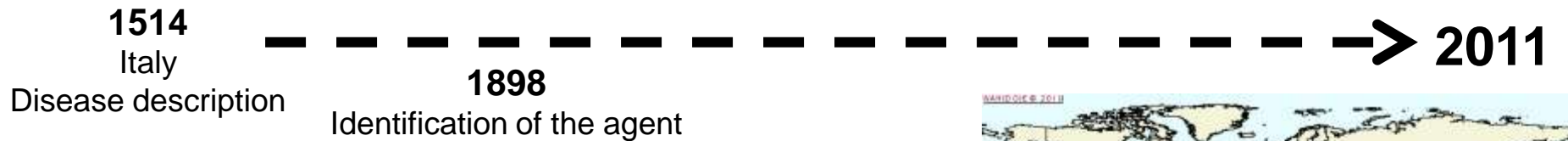




1916 Cattle Diseases, USDA

Foot-and-Mouth Disease (FMD)

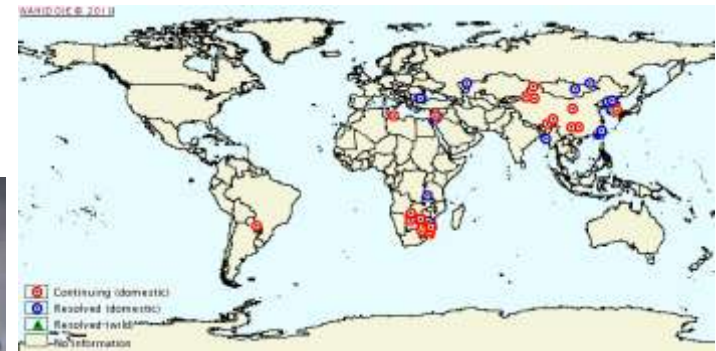
Old disease but actual threat...



UK 2001



JAMES CHAPMAN and KIRSTY WALKER



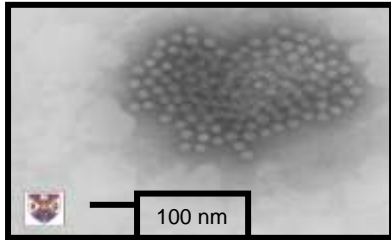
Foot-and-Mouth Disease (FMD)

- **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)



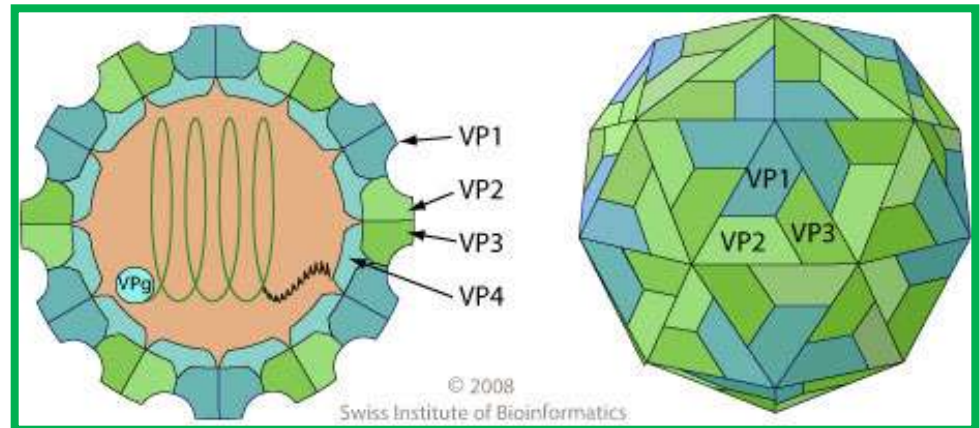
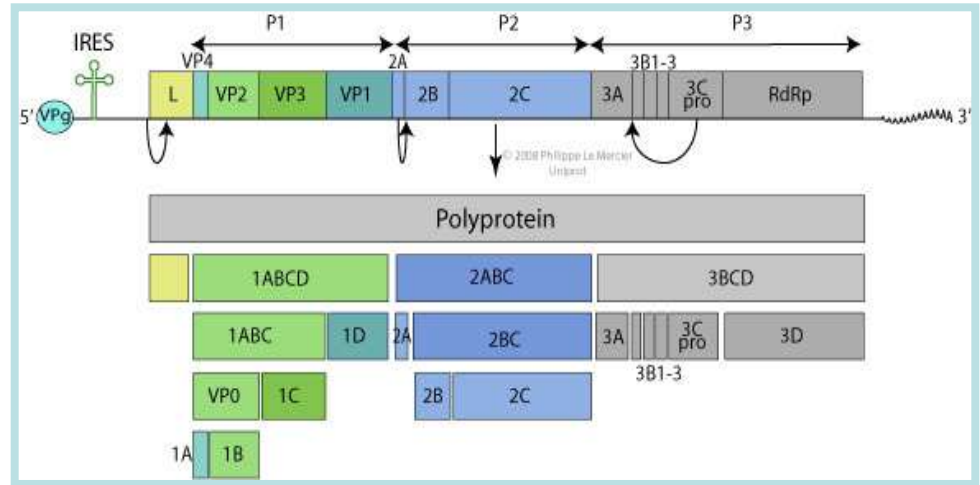
FMDV (Foot and Mouth disease virus)



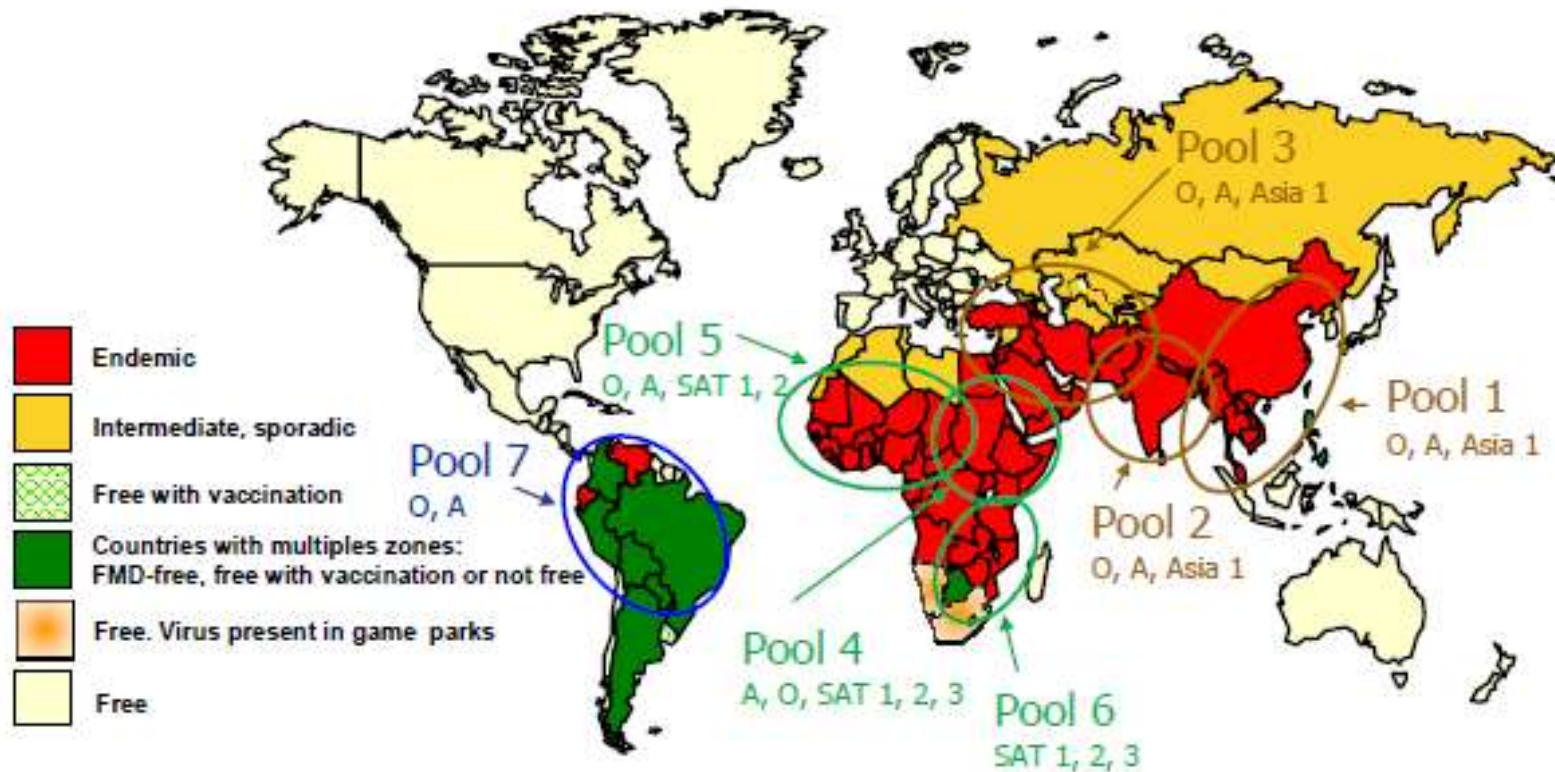
Family : *Picornaviridae*

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 €



Moutons et bovins incinérés dans le centre de la France. ©AFP François Nascimbeni



clabedan-typepad-com



Spread of FMDV (Japan)

**Japan, April-August 2010
(Miyazaki)**

-Serotype O-

292 Outbreaks

211 608 animals destroyed

3.14 billion USD



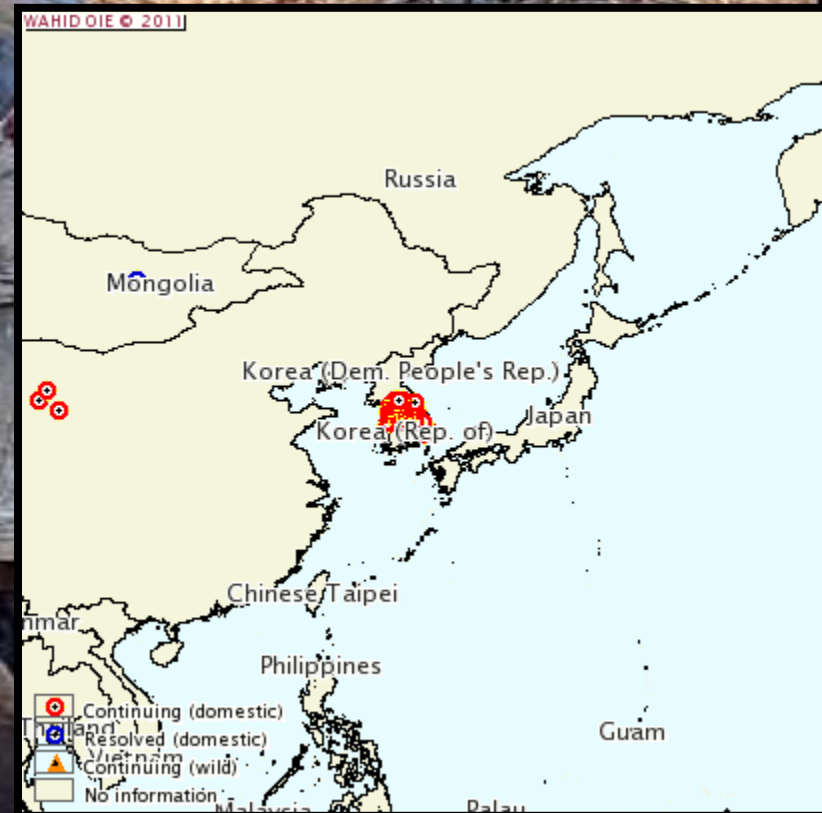
Spread of FMDV (Korea)

**South Korea ,
Beginning: Late Nov 2010**

-Serotype O-

**175 outbreaks
3.3 millions Animal Destroyed
1.8 billion USD**

Vetsweb.com



Bulgaria (near the border with

First

**! Free countries !
are vulnerable to FMD**

12 Outbreaks
1372 Animals Destroyed



Control of Foot-and-Mouth Disease

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.

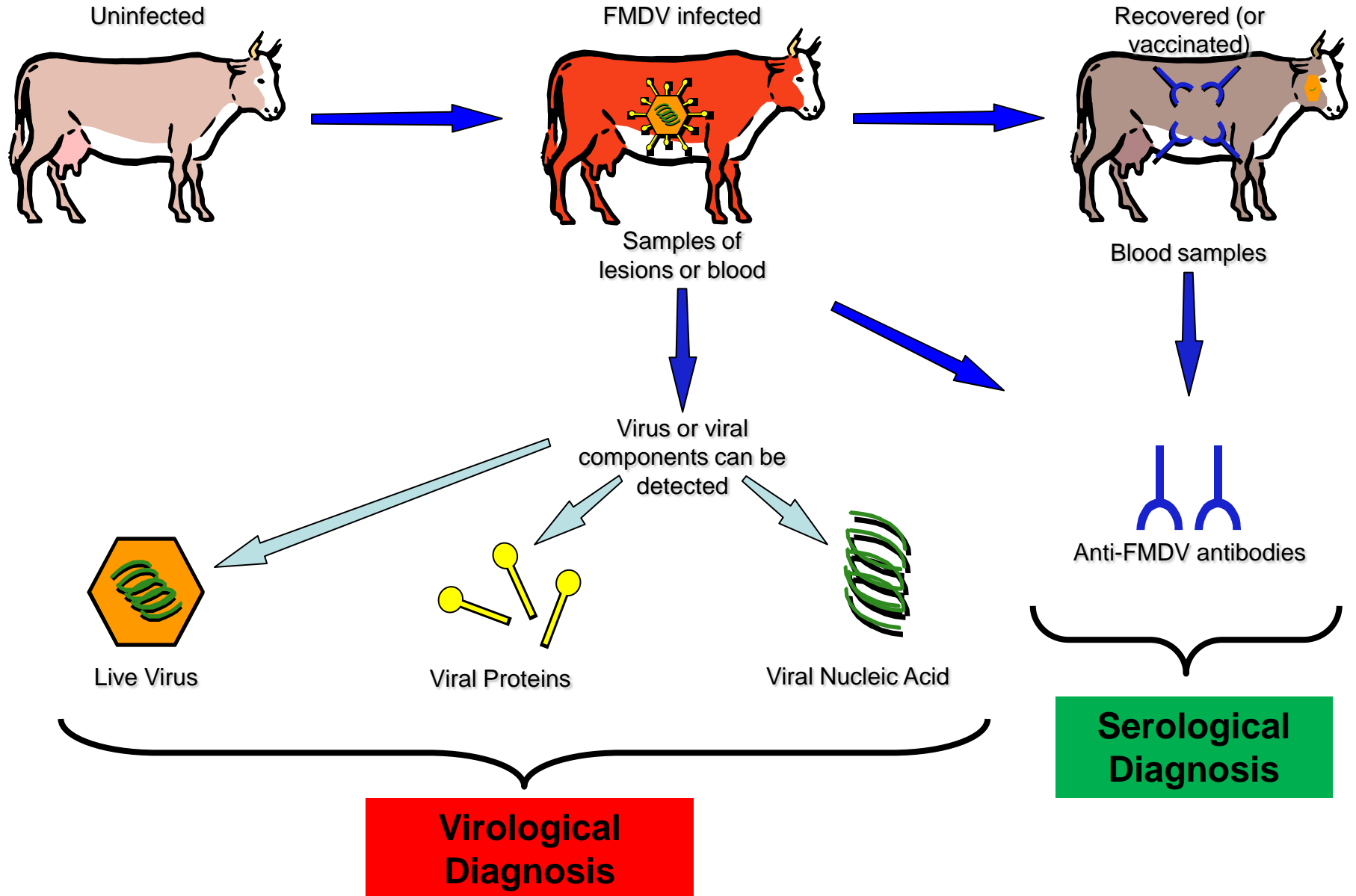
Diagnostic Laboratory



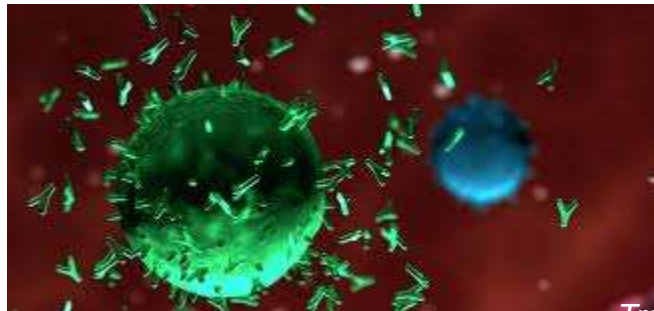
Role of Diagnostic laboratory

- 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

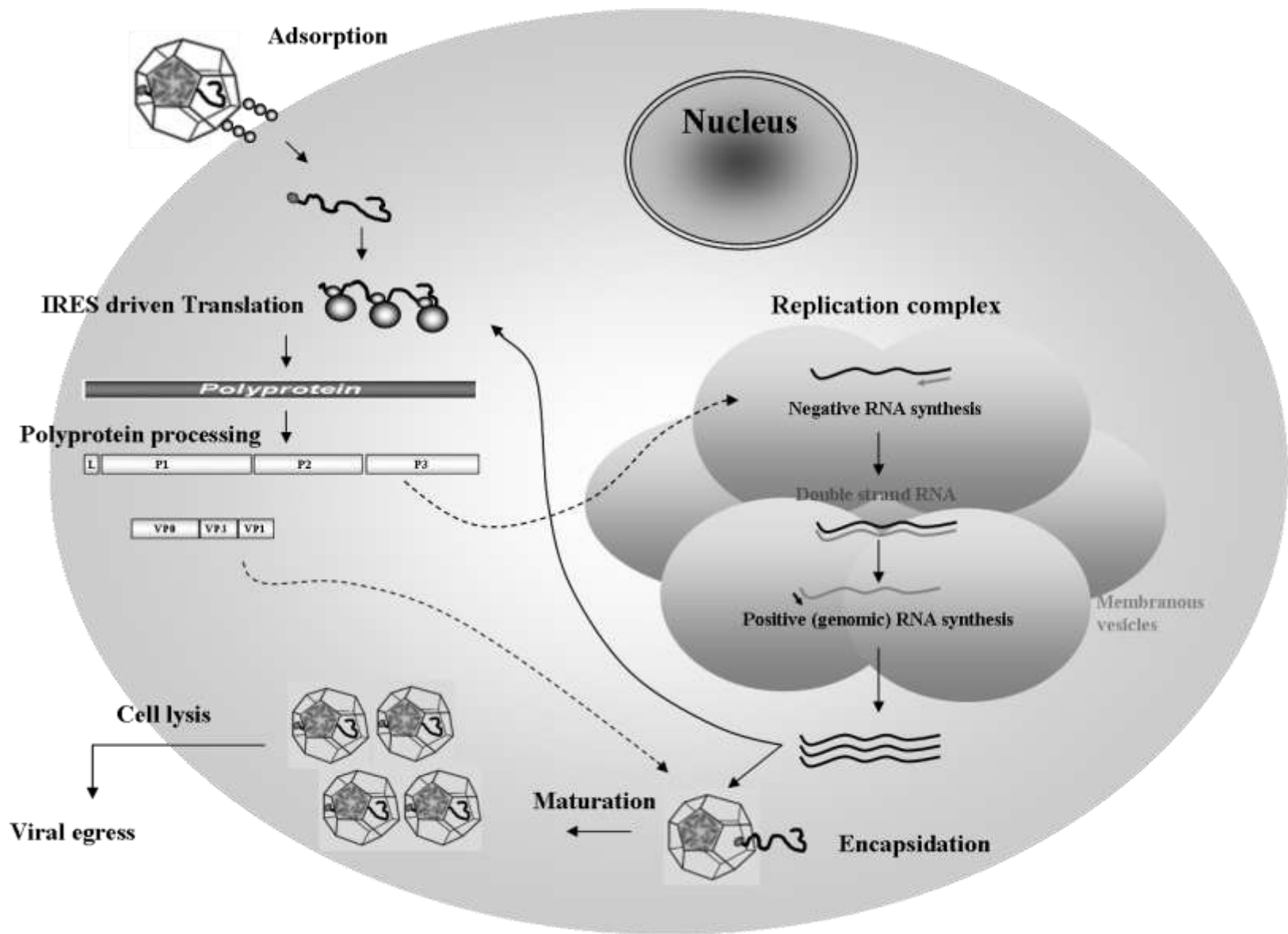
FMDV Diagnosis



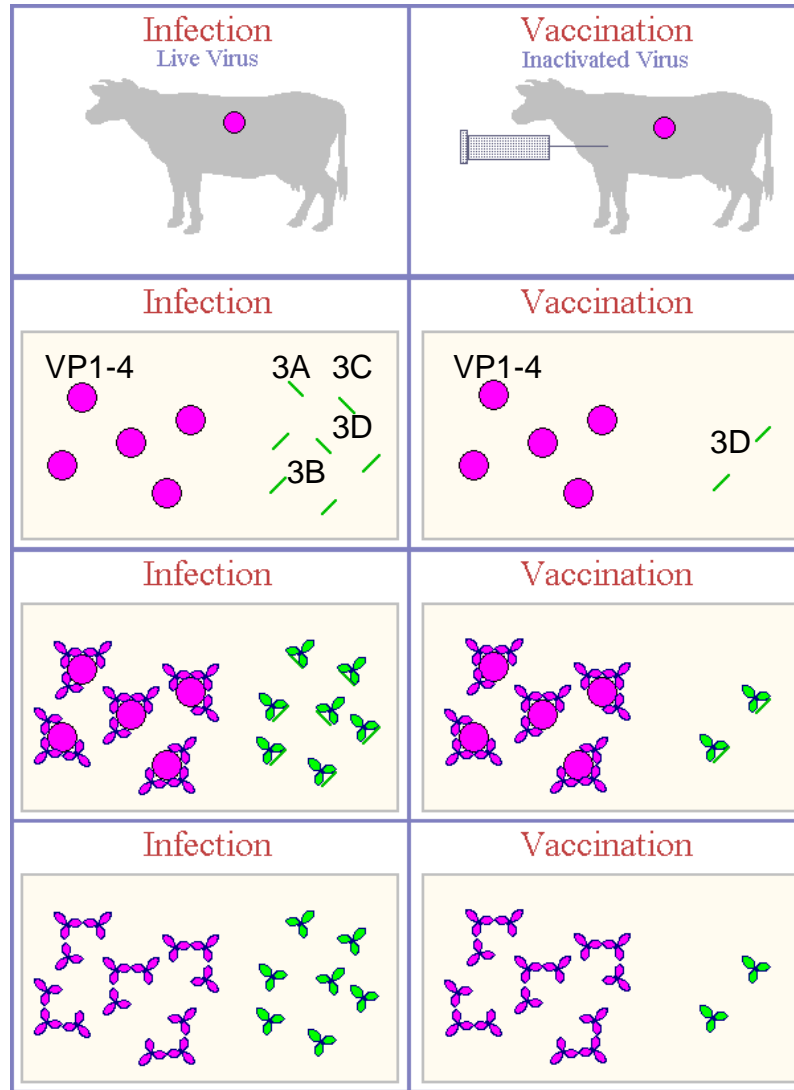
SEROLOGICAL DIAGNOSIS OF FMD



Replication cycle of the virus



Antibody response in animals



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.

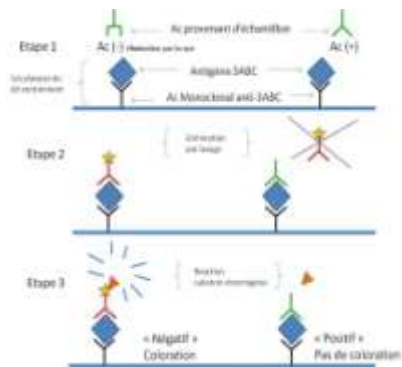
Vaccinated animals produce antibody primarily to structural (capsid) proteins and respond only weakly to contaminating non-structural proteins.

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

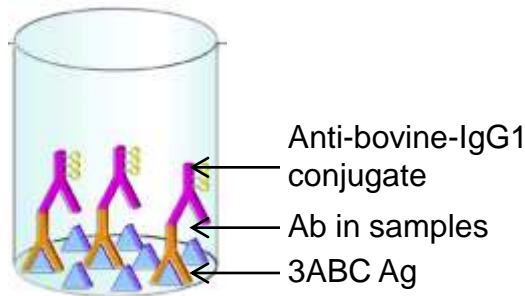
Serological Methods

Antibodies against NSP

Kit Priocheck NSP



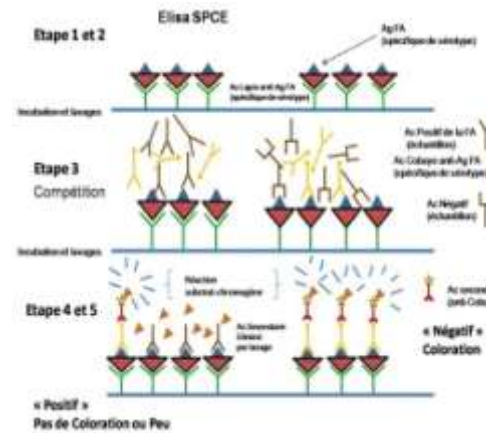
SVANOVIR® FMDV 3ABC-Ab ELISA



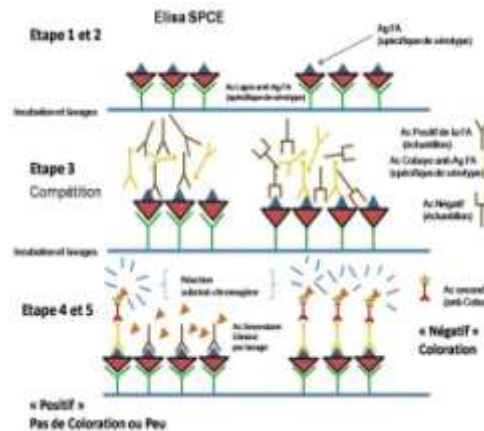
For any Serotype

Antibodies against SP

SPCE

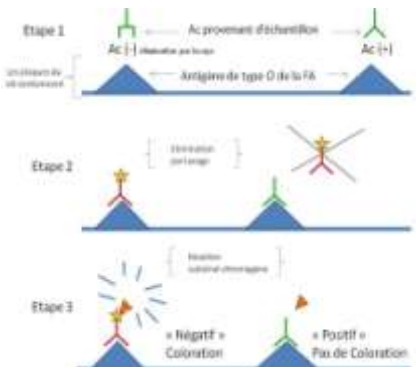


LPBE



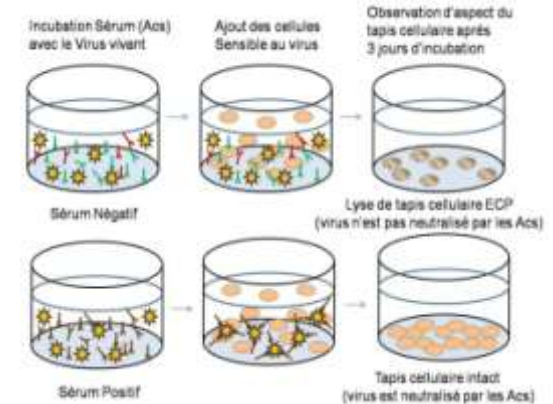
Serotype specific

Kit Priocheck Type O



Virus Neutralization Test

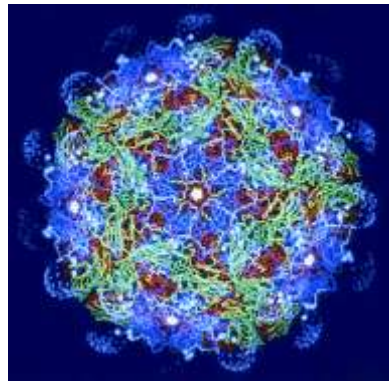
Séroneutralisation



Application

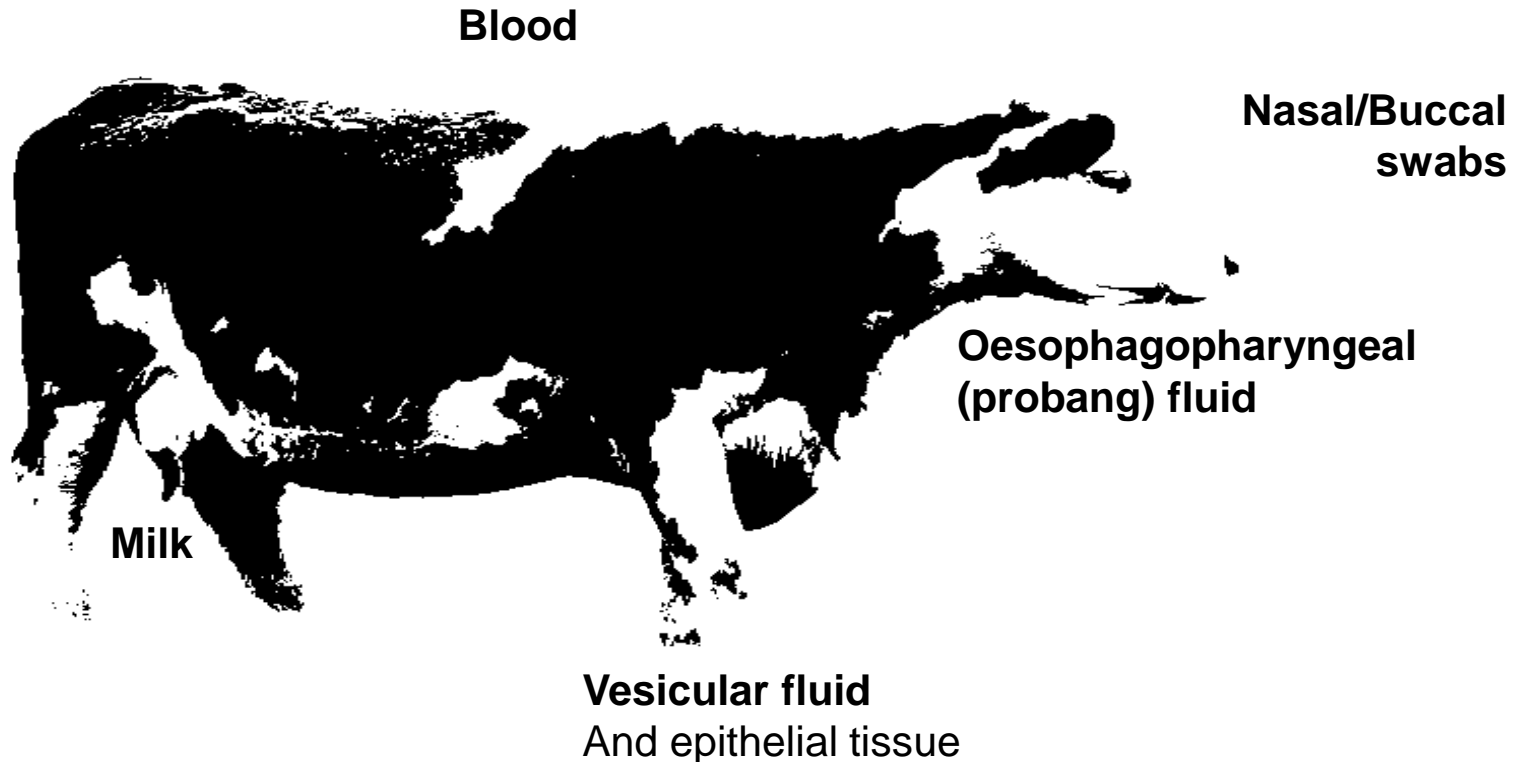
- 1- to certify individual animals prior to import or export
- 2- to confirm suspected cases of FMD
- 3- to confirm freedom following outbreak
- 4- to differentiate infected from vaccinated animals
- 5- to demonstrate the efficacy of vaccination

VIROLOGICAL DIAGNOSIS OF FMD



FMD virus detection

Where to find the virus ?



Also in Heart and other organs of fatal cases

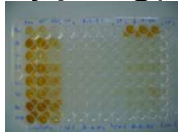
Methods for FMDV detection and typing

Antigen detection

- Virus isolation on cell culture (detection)



- Indirect sandwich ELISA (detection and typing)



- Lateral flow device test (detection)



- Complement fixation test (detection and typing)

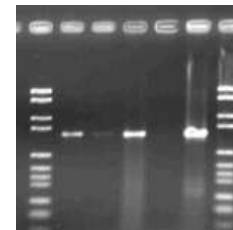


Genome detection

- Real time RT-PCR (detection, typing)



- Classical RT-PCR (detection, typing and molecular epidemiology)



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

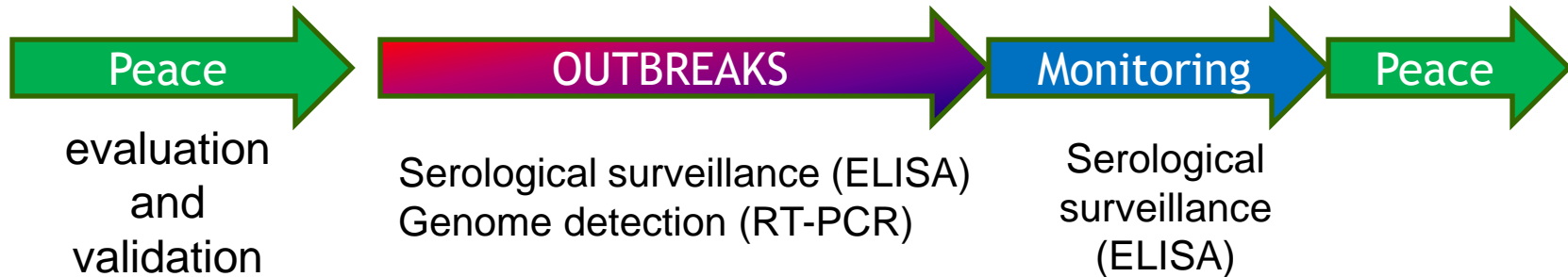


Missions of NRL

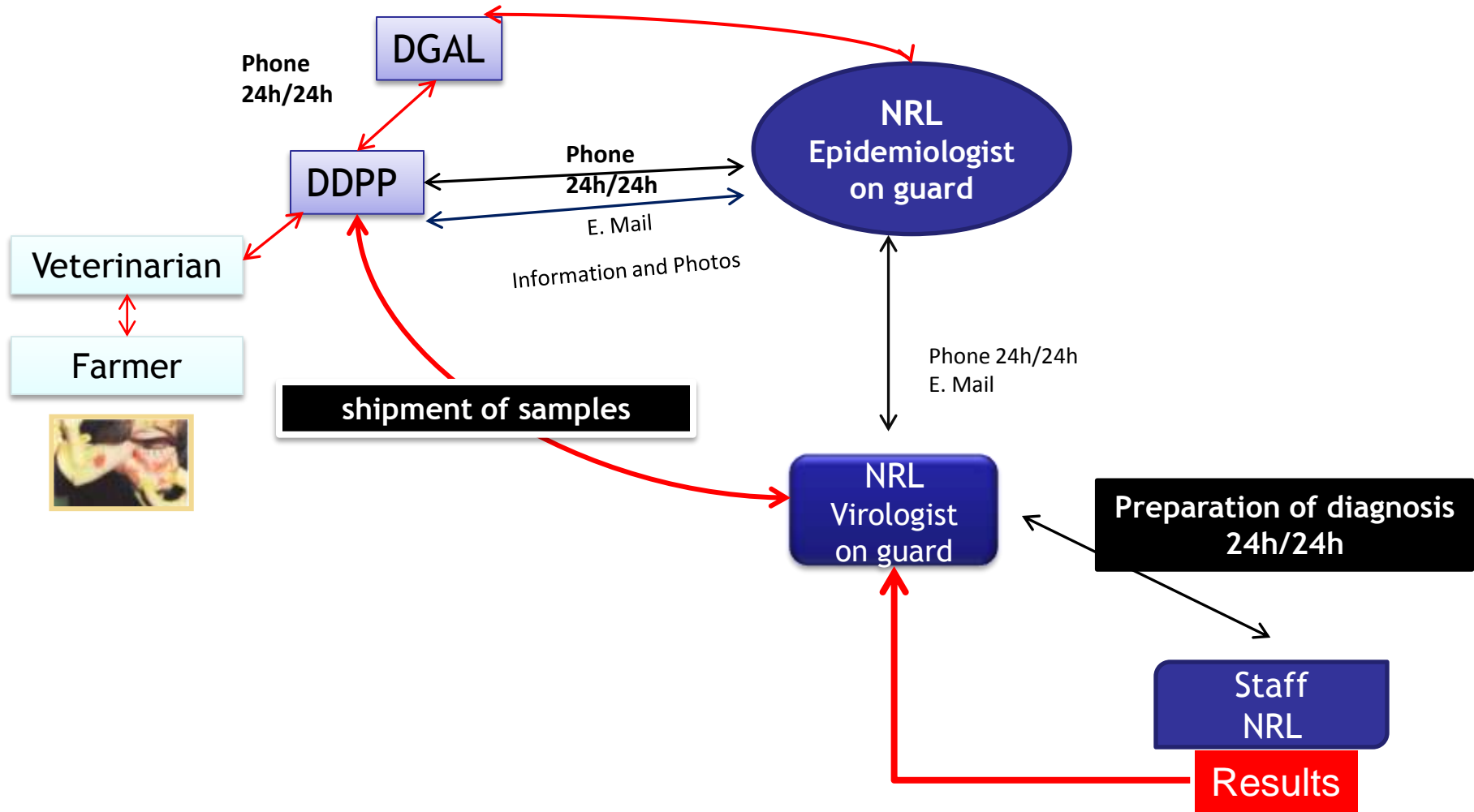
- Provide the Ministry of Agriculture, expertise and technical support,
- **Perform the first-line analysis of suspected cases of FMD,**
- Develop, optimize and validate analytical methods and participate in their normalization,
- Evaluate diagnostic tests,
- Collaborate with the World Reference Laboratory 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

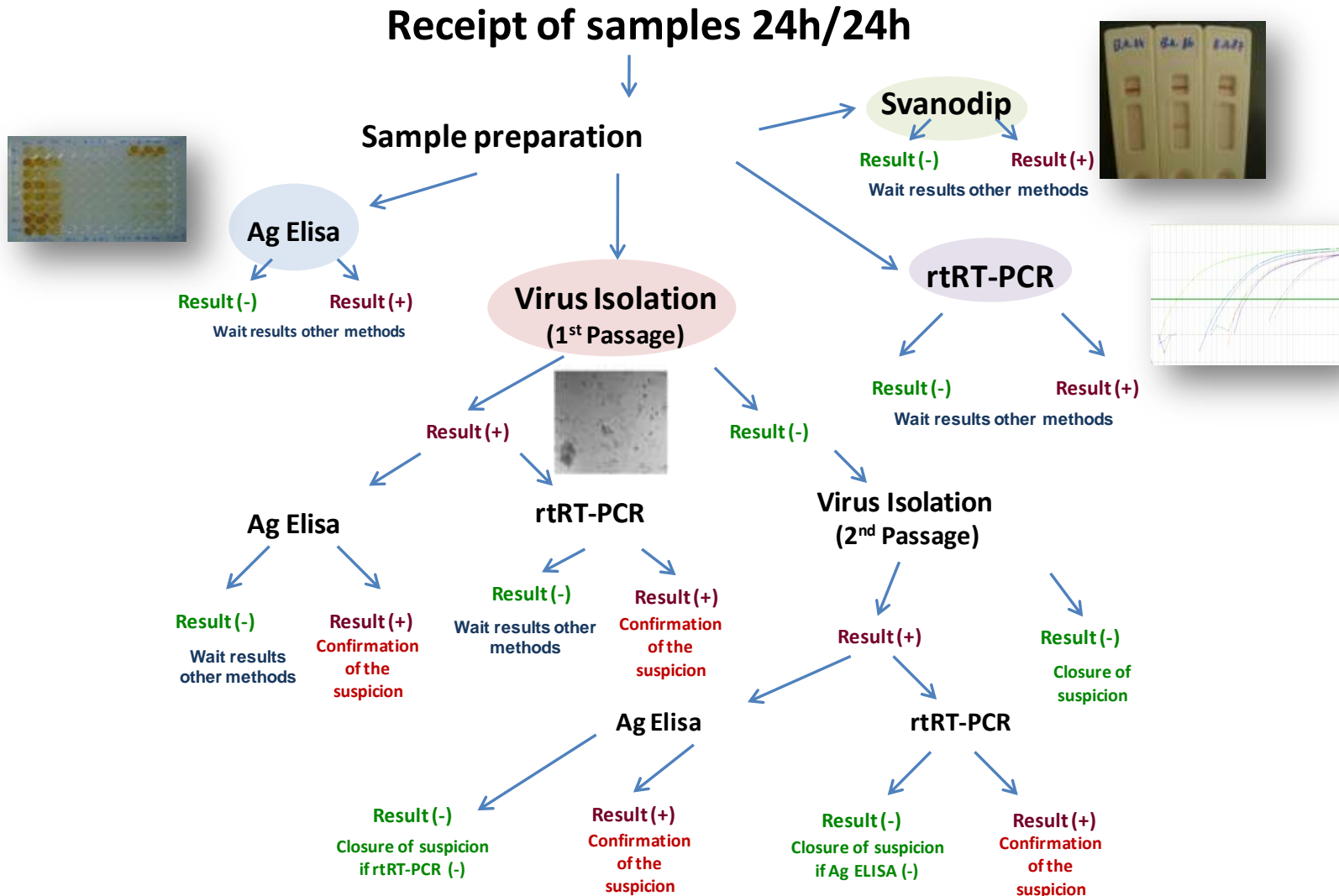
- Participate in the evaluation and validation of serological methods.
- Provide support for serological investigations (ELISA) and virus detection (RT-PCR) during outbreaks.
- Provide support for serological surveillance (ELISA) after slaughter or vaccination.



warning system in France



FMDV detection and typing : Decision tree



FMDV detection and typing in an emergency situation

(Fr NRL training)

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

Suspicion and samples registration

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 :

Personnels	Date et heure	Nom et Visa
Société de livraison : <u>Labo FA</u>	07/02/11 à 9h00	Labo FA
de l'accueil : <u>Labo FA</u>	07/02/11 à 9h00	Labo FA
du laboratoire : <u>Kamila Gorna</u>	07/02/11 à 9h00	KG

Conditionnement des prélèvements :

Etat du colis	Conformité		Observations
Aspect général	Oui <input checked="" type="checkbox"/>	Non <input type="checkbox"/>	
Triple emballage	Oui <input checked="" type="checkbox"/>	Non <input type="checkbox"/>	
Récipient	Oui <input checked="" type="checkbox"/>	Non <input type="checkbox"/>	Tubes en plastique mal étiquetés

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

FMDV detection and typing in an emergency situation

Sample Processing: grinding in sterile sand with a sterile pestle and mortar
with small volume of cell culture medium + antibiotics

Lambeaux d'épithélium/Date et heure de début de traitement : 9H15

lèvement	Conditionnement/poids		Poids utilisé	Volume de dilution	Surnageant après centrifugation	Surnageant utilisé	Surnageant stocké
	Sec	Milieu de transport					
	NA	pH : 7,5	0,77g	1/5 + 3,08 ml	NA	2650µl	1350µl
	NA	pH : 7,5	1,05g	1/5 + 4,2 ml	NA	2650µl	430µl
	NA	pH : 7,2	0,50g	1/5 + 2ml	NA	2650µl	0

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

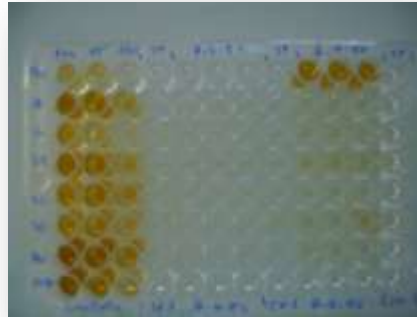
Samples (10h25)

LFD (Svanodip) St 10h25



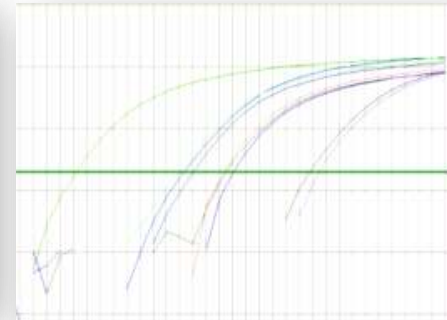
10h35 **S1 (+)**
10h55 S2 doubtful and S3 (-)

Ag ELISA St 10h45



14h30 **S1 (+) type O**,
S2 and S3 (-)

rtRT-PCR St 10h45



15h30 **the 3 samples (+)**

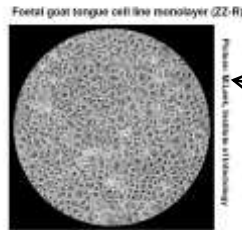
Virus isolation St 11h

	IRES	3D
S1	25,28	29,02
S2	35,44	28,26
S3	34,77	28,41

FMDV detection and typing in an emergency situation

Analysis

III. Morphology of foetal goat tongue cell line



Foetal goat tongue cell line (ZZ-R 127)

Virus isolation
start 11h



Swine kidney cell line (IBRS-2)

	4 PM		6 PM	
	ZZ	IBR	ZZ	IBR
S1	+	-	++	+
S2	-	-	+	-
S3	-	-	+	-

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