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# Foot-and-Mouth Disease and Classical Swine Fever

The impact of EU research (1998-2004)

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# Foot-and-Mouth Disease and Classical Swine Fever

The impact of EU Research (1998-2004)





## Foreword

As Director of EU Biotechnology, Agriculture and Food Research, it gives me great pleasure to present to you the findings of the review exercise of research into foot-and-mouth disease (FMD) and classical swine fever (CSF) as funded by the European Union under the Fifth and Sixth Framework Programmes for Research and Technological Development.

Under the Fifth Framework Programme (1998-2002) – Key Action 2 'Control of infectious diseases' – and under the Sixth Framework Programme – 'Scientific Support for Policies' -, a total of nine projects have been supported. For this publication, which is the tenth in a series, the Commission invited the nine co-ordinators of the projects addressing aspects of FMD and CSF research to present their results to a selected audience in Brussels.

These projects represent an impressive bank of research results which will go a long way in addressing important aspects of control and prevention of these important animal diseases which have had significant economic and social implications for rural communities in Europe. In addition, it addresses the new aspects in the EU animal health legislation on both diseases and the activities of the relevant international organisations.

While this particular review exercise represents a specific targeted area of research, there is no doubt that the different reviews have been a great success and have helped us to concentrate and focus on future research needs and recommendations for improving EU funded research from both technical and administrative sides. Indeed, strategies for the seventh framework programme are currently being developed and debated across the European Union and this publication can be viewed as an important input to the process of drafting suitable future research topics for coverage under the annual work programme. In addition the results themselves have created a significant baseline upon which further research developments in FMD and CSF can be pursued.



Christian Paternmann  
*Director of Biotechnology,  
Agriculture and Food Research*



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## About the workshop

Recent outbreaks of foot-and-mouth disease (FMD) and classical swine fever (CSF) have caused enormous economic damage in the EU. However, these – and other epizootic diseases, like avian influenza – are very much a global problem. Their effective control and prevention, as well as research in this field, require an international approach. This workshop brought together scientists and policy-makers from all over the world. The aim was to strengthen international ties and ensure that the EU remains among the leaders of international initiatives.

### Advertising EU research

Why a workshop specifically on FMD and CSF? First of all, in developing countries these two diseases hinder sustainable development, food security and fight against poverty – the mainstays of EU development policy. The outbreak of CSF in the EU in 1997/98 and of FMD in 2001 cost billions of euro and several millions of animals were killed or destroyed. The control measures applied in these recent outbreaks raised ethical questions regarding the mass culling of animals. They also had a marked social and environmental impact, brought to the public's attention by extensive television and newspaper coverage.

European research has contributed to the adoption of the latest scientific developments not only in EU legislation but also in international standards. Collaboration in EU projects has led to strong links between European laboratories and to several outside the Union. The EU has funded research into FMD and CSF and other epizootic diseases of livestock in both the Fourth and Fifth Framework Programmes (FP4 and FP5). In FP6, a number of new projects have been initiated on FMD and CSF.

### Extending co-operation

The aims of the workshop were primarily to review the results and progress of on-going EU projects and to set targets in future projects. It also examined further needs for research and the



possibilities available in current programmes. The meeting addressed activities carried out by non-EU countries and organisations, as well as the extent of international co-operation in this area.

Participants came from various backgrounds:

- Administrators and decision-makers from the European Commission and Chief Veterinary Officers from Member States.
- Researchers from the EU and third countries (Argentina, Australia, Brazil, Canada, India, China, Switzerland and the United States).
- International organisations (OIE, FAO, ILRI).
- Scientific advisory bodies.
- Farmers' representatives.
- Industry representatives.
- A Member of the European Parliament also attended.

The workshop provided participants with a unique opportunity for networking and extending future co-operation.



## Legislative action



**An overview was given at the meeting of the new legislation regarding the control of CSF and FMD, appearing in Council Directives 2001/89/EC and 2003/85/EC respectively. New items cover mainly restrictions on the movement of animals and products, and guidelines for contingency plans and emergency vaccination.**

The objectives of the Community legislation are immunisation from the diseases and infections without practicing prophylactic vaccination. Four main principles govern the EU policy:

- the responsibility lies with Member States.
- the coordination is ensured by the Commission.
- the measures are flexible to reflect different situations.
- the decision-making process needs to be transparent.

The recommendations laid down in the EU policy reflect changes in the Terrestrial Animal Health Code and the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health (OIE).

The amendments introduced into the aforementioned directives take into account the latest scientific developments in disease control, the experience gained during the recent outbreaks and the recommendations laid out in the opinions of the Scientific Committee of Animal Health and Animal Welfare, as well as the evolution in production systems, in particular the development of densely populated livestock areas and increased trade.

In addition, EU legislation establishes measures to prevent entry of the disease through the introduction of contaminated products of animal origin, by setting up of border controls, inspections and assistance to neighbouring countries. It foresees prepared measures

including notification and monitoring, the establishment of contingency plans by Member States, the designation of diagnostic reference laboratories in each Member State and at Community level, and the establishment of antigen, vaccine and reagents banks.

The measures to control the diseases include stamping out, preventive culling, zoning and regionalisation, movement control of animal and products, cleansing and disinfection, emergency vaccination, surveillance and controlled restocking.

As regards FMD, Council Directive 2003/85/EC introduces new elements, such as: the definition of outbreaks including special cases, suspect restriction areas, detailed restriction on the movement of animals and products, active surveillance for lifting restrictions, specific provisions for the antigen and vaccine bank,



guidelines for contingency plans crisis units, experts groups, decision on emergency vaccination diagnosis, and the definition of densely populated livestock areas. The new Directive also introduces rules and principles for the control of FMD in wildlife. An important element is that trade between Member States in vaccinated animals remains forbidden.

The new Directive takes account of the latest scientific developments in diagnosis (ELISA, PCR, standards), in genetic and antigenic characterisation of virus isolates for epidemiological studies and selection of vaccines, and the detection of non-structural proteins allowing the detection of infected herds in a vaccinated population.

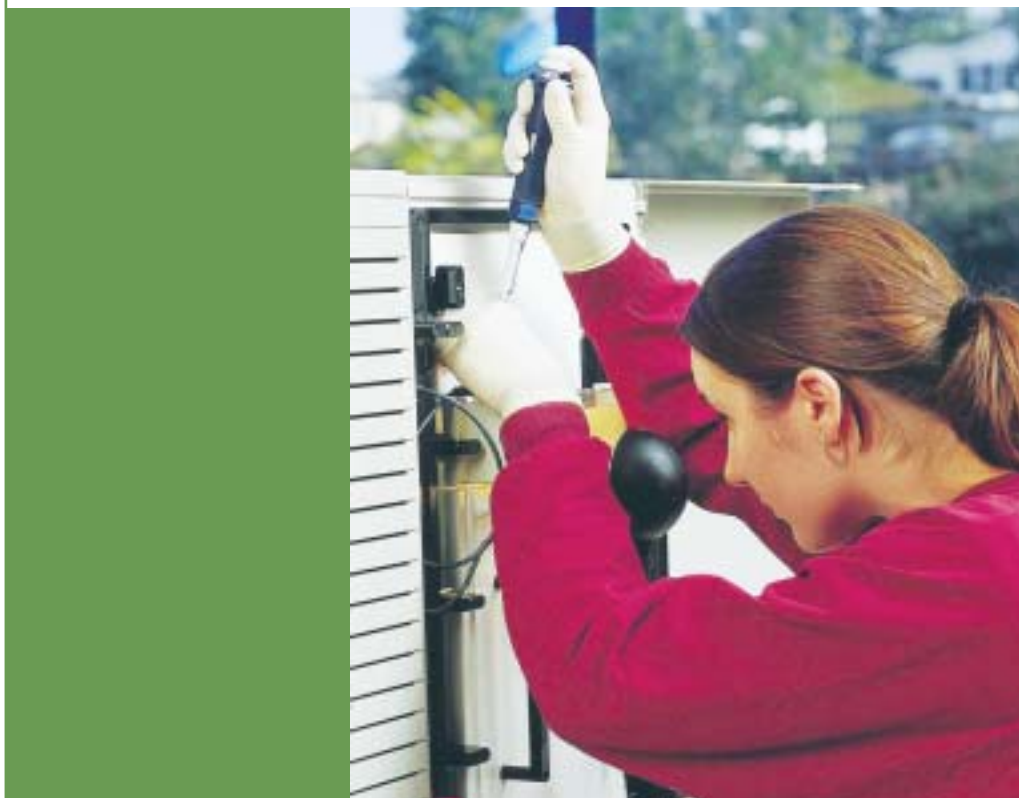
With regard to CSF, Directive 2001/89/EC contains the same basic elements with additional measures accounting for the specific epidemiological features and problems with this disease in the EU, in particular its occurrence and persistence in wild boars and the risk of its spreading to domestic pigs. It foresees disease-control strategies aimed at reducing the number of non-immune wild boars and favouring the establishment of herd immunity by vaccination, and

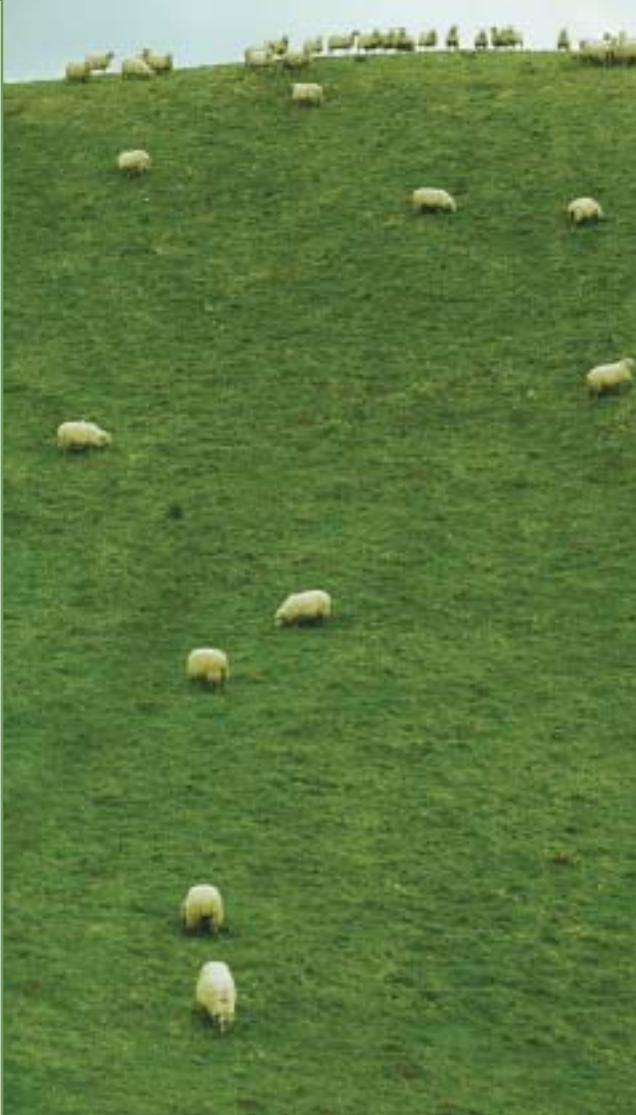
adapted hunting strategies keeping wild boars under control until sufficient herd immunity has developed. In all cases, intensive surveillance must be put in place. Another important element is the possibility of using marker vaccines in emergency vaccinations. This has become possible with the development of two sub-unit vaccines accompanied by the corresponding discriminatory test. As in FMD, strict trade restrictions are enforced for vaccinated animals, which could be reduced by the use of marker vaccines.

In both cases, European research has indeed contributed to the evolution of the legislation. Research efforts should be pursued to increase continuously knowledge of these diseases and to improve the tools to fight them.

## European Food Safety Authority (EFSA)

The role of the newly created European Food Safety Authority (EFSA) was explained by Dr Jordi Serratosa, Scientific Coordinator of EFSA Animal Health and Welfare (AHAW) panel. EFSA provides independent scientific advice with a direct or





indirect impact on food safety. It carries out scientific-based assessments of risks affecting the safety of the food supply, including matters related to animal health, animal welfare and plant health. EFSA also undertakes risk communication.

The tasks executed by EFSA are set by legal requirements, at the request of the Commission, the European Parliament, a Member State or on its own initiative.

The priorities the AHAW panel has set for itself relate to control of FMD and CSF and include:

- a review of diagnostic tests and vaccines, and other tools, for the eradication of diseases on the OIE list A;
- monitoring of movements of animals and the structure of production. This covers the health status of imported herds and on-farm diagnostic testing;
- the impact of different rearing systems on multi-factorial diseases;
- the impact on animal health and welfare of different kinds of dairy cattle management.

#### **Contacts**

[http://europa.eu.int/comm/food/animal/index\\_en.htm](http://europa.eu.int/comm/food/animal/index_en.htm)

#### **Jordi Serratosa**

European Food Safety Authority

Largo N. Palli 5/A

Parma 43100

Italy

E-mail: [Jordi.serratosa@efsa.eu.int](mailto:Jordi.serratosa@efsa.eu.int)

<http://www.efsa.eu.int/>



## Global collaboration

**The pathogens that cause epidemic livestock – and human – diseases respect no frontiers. Carried by aircraft and cargo ships, diseases such as foot-and-mouth-disease (FMD), classical swine fever (CSF), avian influenza and severe acute respiratory syndrome (SARS) can spread quickly across country borders. Effective prevention and control requires tackling them at source, which means close international co-operation. As emphasised at the meeting, the EU actively supports both research and policy partnerships at international level.**

Several projects within the Sixth Framework Programme address issues relating to transmissible diseases. Each one tries to involve all relevant stakeholders, researchers, policy-makers, international organisations and non-EU countries. This ensures that Europe participates in international research initiatives at all levels. In this spirit of international co-operation, the main objectives of this workshop on FMD and CSF concerned the following topics:

- Assessing the activities of countries outside the EU and international organisations and the extent of mutual co-operation;
- Examining possibilities for improving co-operation at international level;
- Facilitating interaction among scientists, industry representatives and decision-makers from both EU and third countries.

The meeting gathered together many of the leading voices in FMD and CSF from the EU and the rest of the world. Members of the World Organisation for Animal Health (OIE), the Food and Agriculture Organisation (FAO), the European Commission Health and Consumer Protection, Development and Research Directorates (SANCO, DEV and RTD DGs), as well as the Director of the Australian Animal Health Laboratory, all gave presentations on the need to work together to be really effective at a global level.

### OIE

The OIE is the international scientific organisation principally concerned with matters related to animal health of terrestrial and aquatic animals, including zoonoses. OIE monitors and informs about the status of animal disease worldwide. Each of 167 member countries reports information about animal diseases on its territory to the OIE, which then passes it on to other countries. Countries are then in a better position to take the necessary preventive action. This information is communicated mainly via the OIE website, e-mail correspondence and two periodicals – *Disease Information*, published weekly and *World Animal Health*, which appears annually.



OIE sets standards for the animal health conditions governing international exchange of animals and for diagnostic tests and vaccines for animals which are recognised as official international references by the World Health Organisation. The standards are drawn up by working groups that include experts from the network of over 150 collaborating centres and reference laboratories – including those in the EU. The latest scientific advances are constantly incorporated into the code so as to improve animal health worldwide and to promote and preserve the safety of international trade in animals and animal products.

This same OIE network of centres collects, analyses and disseminates the latest scientific information necessary to upgrade the methods used to keep these diseases under control. The network also organises workshops, seminars and conferences throughout the world to provide training and to facilitate close collaboration amongst scientists.



The OIE representation in Europe comprises 49 member countries, including all EU Member States. In fact, the EU both contributes to and benefits greatly from this vast network of laboratories and scientific institutions. It provides a firm base for the implementation of research projects, particularly those relating to FMD and CSF, but also avian influenza.

In his presentation at the workshop, '*The OIE and international co-operation in veterinary science for the control of infectious animal diseases*', Dr Alejandro Schudel, Head of the OIE's Scientific and Technical Department, emphasised the close working relationships that exist between the OIE, FAO, WHO and European institutions. He described the benefits of on EU coordination action in the Sixth Framework Programme where OIE is participating in this close collaboration and coordination of research on the control of infectious animal diseases in Europe and worldwide. A particularly fruitful outcome of this is expected to come from co-operation between the OIE and the European Food Safety Agency (EFSA).

## The role of the FAO

The FAO is the principal organisation in the United Nations system concerned with improving agricultural productivity and nutrition, and thus the lives of rural populations, with a worldwide responsibility for the improvement agriculture, forestry, fisheries and rural development. One of its most important functions is as a neutral forum where developed and developing nations can meet and negotiate agreements. It also acts as a centralised source of knowledge and information, including both human experts and computerised databases. Coordination of applied research is a major aspect of its work. Most of the research is conducted in the countries concerned, and FAO actions often support networks of scientists and link European institutions with global partners.

The FAO coordinates technical actions to promote control of FMD in many regions of the world through the EMPRES programme. It has a long history of working to prevent entry of FMD into Europe and to increase the range of tools available and the technical capability of countries to diagnose and control this disease, via its European Commission for the Control of FMD



(EUFMD). It supports a World Reference Laboratory for FMD in Europe and the networking of FMD laboratories.

A major part of the work done by the FAO's joint division with the International Atomic Energy Agency (IAEA) is the coordination of research, from its agriculture and biotechnology laboratory at Seibersdorf, Austria. Together with partners throughout the world, it has developed and implemented several new diagnostic tests for FMD. The division works closely with the OIE in developing worldwide standards for the validation of diagnostic assays. It also operates an external quality assurance scheme for veterinary diagnostic laboratories in developing countries.

The FAO and OIE collaborate on another initiative, too: *'The Global Framework for Progressive Control of FMD and other Transboundary Animal Diseases (GF-TADs)'*. This aims to strengthen regional alliances against

TADs throughout Europe and the rest of the world. It should benefit Europe by reducing risk of infection, as well as making available a global pool of expertise upon which to draw regarding FMD issues.

## EUFMD

EUFMD, an integral part of the FAO, works closely with the EC Directorates and the OIE in the surveillance and control of FMD. As a technical support unit for surveillance and control programmes, it gives advice to countries, to the EFSA and others on the whole range of FMD issues, and supports research actions to resolve outstanding technical issues. It acts as a bridge between European institutions and countries requiring technical assistance in which the disease occurs, and supports vaccination campaigns in regions at risk. EUFMD prepares various guidelines relating to control of FMD – often endorsed by the EC and OIE – and undertakes actions to prevent the spread of FMD in Europe.



The European programme activities for FMD have resulted in the early introduction of new technologies for control and surveillance of these diseases. For instance, early application of NSP antibody tests in Europe led to their rapid introduction in the Balkans, North Africa, Turkey and the Caucasus region from 1997 onwards. The insights gained from this success have led to further test developments and early uptake of new technologies.

## Contacts

### OIE

#### **Alejandro Schudel**

Head, Scientific and Technical Dept  
OIE

12 rue de Prony

75017 Paris

France

Tel: +33 (0)1 4415 1882

Fax: +33 (0)1 4267 0987

E-mail: a.schudel@oie.int

[www.oie.int](http://www.oie.int)

### FAO

#### **Joseph Domenech**

Head of Animal Health Service-AGAH

FAO

Viale delle Terme di Caracalla

Rome 00100

Italy

Tel: +39 06 570 53531

Fax: +39 06 570 53023

E-mail: joseph.domenech@fao.org

[www.fao.org](http://www.fao.org)

#### **Keith Sumption**

Secretary EUFMD

FAO HQ, Room C-518

Viale delle Terme di Caracalla

Rome 00100

Italy

Tel: +39 06 570 55528

Fax: +39 06 570 55749

E-mail: Keith.Sumption@fao.org

<http://www.fao.org/ag/againfo/commissions/en/eufmd/eufmd.html>

#### **Juan Lubroth**

Senior Officer – Infectious Disease Group/EMPRES

FAO Animal Health Service

Animal Production & Health Division

Viale delle Terme di Caracalla

Rome 00100

Italy

Tel: +39 06 570 54798 and 570 52909

Fax: +39 06 570 53023

E-mail: juan.lubroth@fao.org

<http://www.fao.org/EMPRES>



## Project summaries

Both recently completed and ongoing EU-funded projects within FP5 and FP6 were presented at the Brussels workshop. Research coordinators outlined the objectives of their work and the achievements to date, the key features of which are given here. For further details, see pages 13-29.

### Tests for foot-and-mouth disease (FMD)

Recent outbreaks of FMD have been hugely damaging to national economies in the EU. To help implement better control strategies, improved tests are needed to rapidly trace sources of the disease and sites of new infections. The **FMD diagnosis** project has yielded several diagnostic tools in this area. The real-time polymerase chain reaction (real-time PCR) techniques developed are more accurate and can handle large numbers of samples, while the risk of cross-contamination of samples has also been reduced. A solid-phase ELISA (enzyme-linked immunosorbent assay) has been established and applied for surveillance screening, and other novel tests have been successful in detecting previous infection in vaccinated cattle.

### Simultaneous virus testing

There are many different viruses that cause outbreaks of disease in farm animals, so it is important to identify the pathogen responsible as early as possible. However, conventional methods currently in use are either slow (viral isolation in cell culture) or not sufficiently sensitive (immunological assays). To overcome these shortcomings, the **Multiplex** project aims to detect different viruses simultaneously and very rapidly. They will use a technique termed 'multiplex reverse transcription-polymerase chain reaction' (multiplex RT-PCR) and/or multiplex real-time RT-PCR (multiplex RRT-PCR). The partners have established a virus genetic database, and have produced novel ELISA antibody kits as well as multiplex RT-PCR and multiplex RRT-PCR systems. These systems are actually in operation in five European countries.

### Pig vaccine delivery

The use of marker vaccines for classical swine fever (CSF) allows infected pigs to be distinguished from

vaccinated animals. Their use may reduce the economic impact of an outbreak and mean that many fewer healthy pigs need to be destroyed. The goal of the **Efficacious delivery** project is to find a suitable delivery system for a series of new marker vaccines. The research partners have generated four prototype construct vaccines of which, to date, an E2-expressing vector vaccine has conferred good immunity against CSF. The project team has also developed multiplex RT-PCR tests to measure the immune responses induced.

### Immune response mechanisms in CSF

Understanding how the CSF virus evokes an immune response will help in research on new marker vaccines. In the **IMPCSF** project, the partners will define components of the virus that interfere with the pig's immune defences. They will also define components of the virus stimulating the pig's immune defences. This knowledge can be used to develop better marker vaccines in the future. They will discover which viral proteins and epitopes are involved by examining the activities of cytotoxic T lymphocytes, produced after vaccination and infection, as well as the role of viral B cells and T helper cells. This knowledge is needed in order to make a better marker vaccine. Marker vaccines make it possible to detect infected pigs in vaccinated populations, and thus avoid mass culling of healthy, uninfected pigs.

### Vector-based vaccine for FMD

FMD has the greatest economic impact of all viral diseases in farm animals, but the vaccines that are currently available do not protect against all the different forms of the virus. The **FMDV vaccine** project aims to develop safe and effective anti-FMD vaccines that will also prevent the development of the carrier state. The project partners have constructed a cDNA vector-vaccine based on the transmissible gastroenteritis virus (TGEV), which has demonstrated very promising properties.

### DNA-based vaccines for FMD

DNA vaccines against FMD have several advantages over conventional ones, and are cheaper to produce. The objective of the **FMDna vaccine** project is to find the best DNA vaccine for

use in sheep and pigs. The partners have prepared various virus-derived plasmids and carrier molecules for such vaccines, and several potential adjuvants for them are being evaluated. Experiments are in progress with cationic microspheres on to which the DNA vaccine is adsorbed. Later, the partners will focus on the relative immunogenicity of different FMDV antigens.

### FMD carrier state

The project **FMD Tropism** aims to discover how the virus selectively chooses to reside at particular body sites in infected animals, especially in the upper respiratory tract. Partners will examine expression levels of certain integrins – identified as receptors for the virus – in the upper respiratory tract of cattle with FMD. They hope to determine whether the carrier state is maintained by adaptive changes in the virus or by changes in integrin expression within cells at particular body sites during infection.

### Control of CSF in wild boar

Wild boars harbour the CSF virus and so present a risk of infection to domestic pigs. Partners in the project **CSF vaccine and wild boar** will first devise an epidemiological and economic model for eradication of CSF in the animal. They will then develop a live marker vaccine that offers long-lasting immunity against CSF. This will be administered orally to the animals in the form of baits. New diagnostic tools (RRT-PCR, pen-side and new ELISA tests) will be developed to discriminate between vaccinated and naturally infected animals. The project will also lead to a better understanding of CSF transmission post vaccination.

### Improved control of FMD

EU policy is set to adopt emergency vaccination as an option in the control of FMD. To enable the use of those vaccines that are currently available, new diagnostic tests are required to distinguish between infected and vaccinated animals. Research teams in the **FMD-ImproCon** project will improve existing tests and generate new ones which can do this. Vaccines will be developed to provide mucosal immunity as such immunity staves off local infections and prevents the carrier state from arising. Numerous experiments will provide a wealth of information on the impact of vaccination.



## Diagnosis of viral diseases

Recent outbreaks of viral disease in farm animals in EU countries have required emergency large-scale culling. This has been extremely costly both for the Union budget and for consumers, and has given rise to considerable public concern. The most economically damaging infections have been foot-and-mouth disease (FMD) and classical swine fever (CSF). These have been studied in depth by participants at the workshop.

Effective control strategies have to be introduced as soon as possible, which means catching infected animals early – before they have a chance to infect many others. Tests must be able to distinguish between apparently healthy but infected animals and vaccinated ones, and from others that remain uninfected. This requires improving existing laboratory tests, as well as producing new ones. Towards this goal, modern test methods such as ‘real-time reverse transcription-polymerase chain reaction (RRT-PCR)’ and specific immunoassays are being further developed.

Other requirements for the new generation of tests are:

- rapid and highly sensitive tests to detect disease in animals as soon as possible after they become infected;
- simple and rapid tests for use in the field to affirm or negate suspicions of clinical symptoms;
- sensitive and specific tests to detect infection in an individual animal;
- rapid and sensitive tests for differential diagnosis of diseases that hide similar clinical signs.

## Diagnosis of foot-and-mouth disease (FMD)

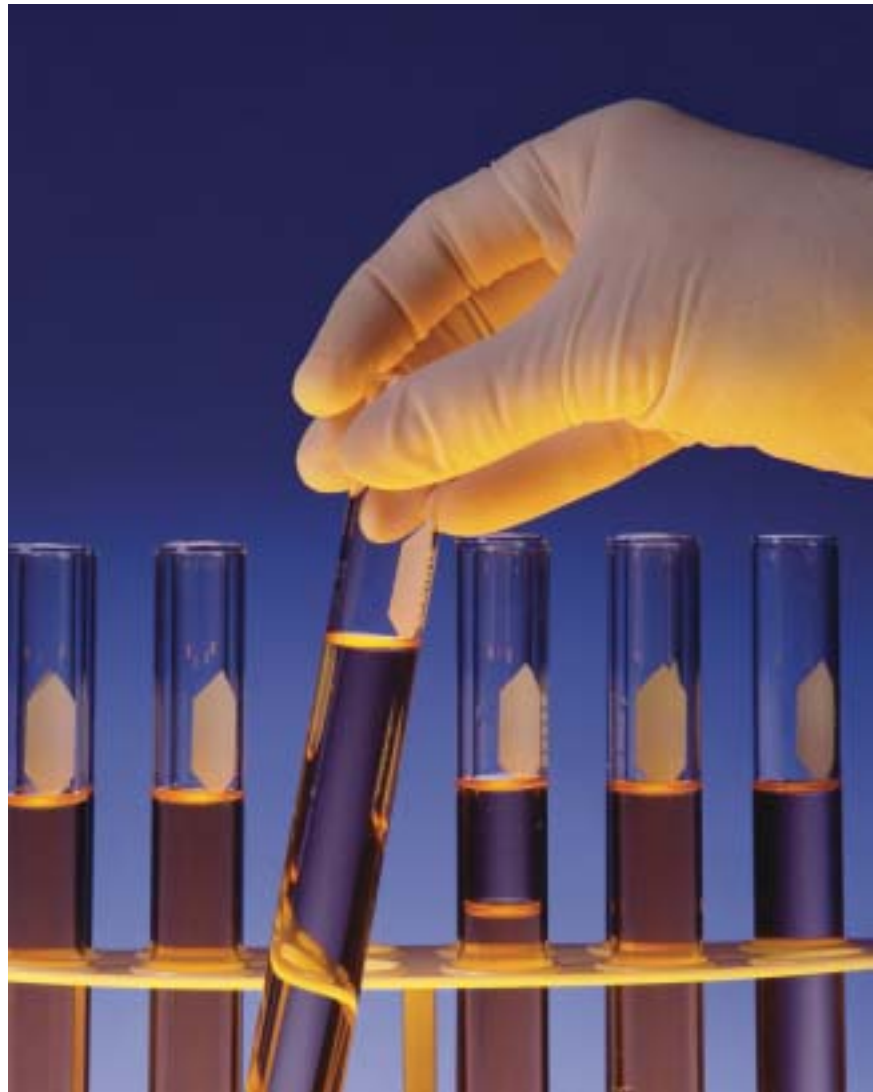
**The massive outbreak of FMD that overwhelmed the UK in 2001 was not detected until several days after the pigs, where the disease was first detected, had contracted it. By that time, infected sheep had been sent from farms to abattoirs across the country, spreading infection. Clearly, better laboratory tests are needed to diagnose the disease. Tests available at that time could confirm the presence of FMD, but what was missing was rapid tracing of sources of the disease and of new infections, as well as proof that individual animals were not infected.**

In the modern world, the free movement and intermixing of animals across borders on their way to market enables diseases to spread very quickly. Once an outbreak occurs, fast and reliable laboratory tests are vital to help halt the disease at its source, and to support the introduction of effective control strategies. If tests are available that can detect infected cows in vaccinated herds, then emergency vaccination becomes a viable option – avoiding the need to destroy large numbers of animals.

### Direct testing

Between 1999 and 2003, the national FMD laboratories in seven EU countries collaborated in a Concerted Action to improve diagnostic methods in this area. They have made significant advances in the detection and characterisation of antigen genomes in the FMD virus. The conventional reverse transcription-polymerase chain reaction (RT-PCR) test, used to amplify and detect viral nucleic acids, had several drawbacks which prevented its use in the 2001 outbreak. The problems included too many false positive and negative results, and the high

risk of cross-contamination when analysing large numbers of samples. The introduction of the real-time PCR has increased both the sensitivity and the specificity of the RT-PCR method, so the proportion of false results is greatly reduced making it more reliable. The improved tests are also less prone to cross-contamination and can be automated to run a large number of samples. Because they target a conserved region of the FMD virus genome, common to all its sub-types and serotypes, they can detect the virus in virtually all its forms.





## Antibody detection

The international partners have also had success with the development of a solid-phase ELISA antibody method for surveillance screening. Such serological screening is required to pick out any animals which excrete the virus due to an unnoticed infection. The new approach is more specific than the liquid-phase ELISA test previously used, and is just as sensitive. In addition, because the virus in the antigen is inactivated, the solid-phase ELISA may be performed in laboratories without special containment facilities.

Tests have also been developed to detect antibodies to infection with live viruses, rather than to the viral proteins used in inactivated FMD vaccines. This is achieved by testing for antibodies to the non-structural proteins (NSP) of the FMD virus. Such tests have proved valuable in detecting previous infection in vaccinated cattle.

Despite substantial progress, more work still needs to be done, especially in the validation of new tests for species other than cattle. New

approaches are being pursued that hold out promise for the future of FMD testing, including a biosensor technology developed by the Spanish partner.

### Contact

#### Bernd Haas

Friedrich-Loeffler-Institut,  
Federal Research Centre  
for Animal Health  
Boddenblick 5a  
D-17493 Greifswald  
Insel Riems  
Germany

Tel: +49 (0)38 351 7-0,

Ext. Dr Haas: -211, -164

Fax: +49 (0)38 351 7-151

E-mail: Bernd.Haas@rie.bfav.de

## Multiple virus testing



Throughout the world, numerous highly contagious viral diseases are able to spread rapidly among farm animals, causing enormous damage in both economic and social terms. Professor Sándor Belák of the National Veterinary Institute and the Swedish University of Agricultural Sciences, Uppsala outlined the significant progress made in bringing new techniques for virus testing into routine laboratory practice.

A consortium of six international partners from Denmark, Hungary, Spain, Sweden, and the United Kingdom are collaborating to develop very fast methods of viral testing. This is known as the **Multiplex** project – development, standardisation and harmonisation of novel multiplex nucleic acid tests for the detection of economically important viruses in farm animals. The main aim is to produce ‘multiplex’ PCR assays – able to detect different viruses at the same time. However, other diagnostic technologies are also being researched within this project. All the viruses targeted are responsible for recent major outbreaks of disease in farm animals worldwide, i.e. African swine fever (ASF), classical swine fever (CSF), Aujeszky’s disease (AD), foot-and-mouth disease (FMD), vesicular stomatitis (VS), porcine reproductive and respiratory syndrome (PRRS), porcine parvovirus (PP), and swine vesicular disease (SVD).

### Conventional methods

The standard laboratory procedure for diagnosis of most viral diseases generally involves isolation of the virus in cell culture – a slow and awkward process. It is also technically demanding and has to be carried out in well-equipped cell culture laboratories. Rapid immunological assays (particularly antigen-ELISA, haemagglutination and immunofluorescence) are available for some viruses, although they are often not sufficiently sensitive.

The methodology and controls used for PCR testing vary from one laboratory to another – in fact, most PCR reagents used are made up ‘in-house’. In addition, conventional PCR can only detect one kind of virus at a time. Hence the need to standardise testing and develop multiplex assays so that reliable testing with a high sample throughput can be performed routinely.

### Results

- **A genetic database** was created for the eight viruses and the data obtained were used in the design of primers and probes for the PCR assays.
- In order to simplify and accelerate the diagnosis, **the viruses were divided into four clusters:** haemorrhagic, vesicular, reproductive and respiratory.



- **Gel-based multiplex RT-PCR methods** were developed and standardised for the detection of hemorrhagic (CSFV/ASFV), vesicular (SVDV/VSV/FMDV) and reproductive (ASFV, CSFV, ADV, PRRSV, PPV) clusters of List A diseases. The assays have been developed to the point of being suitable for routine diagnosis. Work continues to adapt the methodology for the respiratory cluster of viruses.
- **Real-time reverse transcription (RRT-PCR) single assays** were developed for FMDV, VSV and SVDV.
- **Monoclonal antibody ELISA** kits for ADV and PPV have been produced.
- **Controls** for nucleic acid extraction and amplification procedures have been produced.
- **The RRT-PCR systems** are now in operation on real-time PCR machines in various countries (namely, RotorGene2000 in Sweden, iCycler in Hungary, and Stratagene MX4000 in Denmark).

- **A website** ([www.multiplex-eu.org](http://www.multiplex-eu.org)) has been launched by the consortium to provide up-to-date information.

#### Contact

##### Sándor Belák

National Veterinary Institute  
Swedish University of Agricultural Sciences  
Uppsala, Sweden

Tel: +46 18 674 135

Fax: +46 18 647 669

E-mail: [sandor.belak@sva.se](mailto:sandor.belak@sva.se)







## Pig vaccine delivery



The delivery system is a recombinant protein vaccine constructed from the bacterium *Pseudomonas aeruginosa*. The viral vector expresses the CSFV protein E2, while the nucleic acid (cDNA and mRNA) vaccines encode two CSFV proteins – NS3 and E2. Both proteins will be used to protect the animal. The team subsequently modified the nucleic acid vaccines by combining them with DNAs encoding various cytokines.

### Solid protection

**Live virus vaccines are available for the prevention and control of classical swine fever virus (CSFV) in pigs. These are relatively safe and effective, but it is not possible to distinguish between post-vaccinated pigs and those with an infection. Marker vaccines, with their accompanying diagnostic tests, able to reveal this difference are crucial to the prevention of CSFV infection and its spread in the EU pork industry. In this regard, the development and registration of the E2-subunit dead vaccine is a major step forward. However, further efforts need to be pursued.**

The use of marker vaccines to combat outbreaks of CSFV can mean the destruction of far fewer pigs and less farms to be closed down. Such vaccines may also shorten the duration of an outbreak and so maintain morale within the farming industry, as well as making enormous cost savings. Seven European partners in six countries undertook a project to develop and evaluate a series of new CSFV marker vaccines to identify those offering good protection against CSF. The **Efficacious delivery** project, Identification of efficacious delivery systems for recombinant and nucleic acid construct vaccines, began in 2001 and ends in 2005. The team initially generated four prototype vaccines for further research. These comprise a bacterial lipoprotein-based delivery system, one viral vector, and two nucleic acid vaccines.

Experiments were performed on pigs to assess the strength of the immune responses induced by each of these four trial vaccines, and how quickly they began to protect the animals from infection. DNA vector vaccines encoding E2 and/or NS3 were shown to be only partially protective. The RNA and the bacterial-based vaccine did show promise, but failed to confer adequate immunity in follow-up experiments. As part of the project, specialised PCR cytokine assays were developed to measure the strength of the immune responses generated.

The parapoxvirus-based E2 expression was as potent as the modified live CSFV vaccine (gold standard). It was the only vaccine candidate that conferred protection even after a single shot application. The inherent immuno-stimulatory ability of this vector resulted in optimised foreign antigen processing as demonstrated by detailed analysis of immune reactions. Overall, the possibility of safe and efficient intervention marker vaccination offers hope of reducing mass slaughter and has a clear positive influence on the European population.

#### Contact

##### Mathias Büttner

Federal Research Centre  
for Virus Diseases of Animals  
Institute of Immunology  
Paul-Ehrlich-Str. 28  
72076 Tübingen, Germany  
Tel: + 49 7071 967 255  
Fax: + 49 7071 967 303  
E-mail: mathias.buettner@tue.bfav.de

## Understanding the CSF virus



**The rapid development of new, effective and safe vaccines against CSFV requires an understanding of how the virus works. This entails finding out which viral components are involved, and to what extent, when a vaccine successfully induces an immune response.**

A better understanding of immune mechanisms at a molecular level can lead to the creation of new marker vaccines and diagnostic tools for CSFV. These, in turn, will enable better control strategies to be put in place during disease outbreaks or eradication campaigns. Therefore, a Dutch-led team of four European veterinary research institutes has launched the project **Immunological mechanisms of protection against classical swine fever virus: Towards the development of new efficacious marker vaccines (IMPCSF)**. They are investigating the role of viral components important in the early stages of protection, before the first clinical signs of disease appear.

### Removing viral activity

To characterise the immune response against CSFV, project partners are studying the parts and functions of the virus that interfere with a pig's immune defences. The effects of both innate and acquired immunity are being considered. Once the specific components of the virus have been identified, it should be possible to eliminate their immunosuppressive activities from future

vaccine candidates. Meanwhile, components that stimulate the immune defence mechanisms will provide insight into which components should be incorporated in a marker vaccine.

Partners will try to identify and quantify populations of cytotoxic T lymphocytes – natural immune defences that attack the virus – produced after vaccination and infection. The role of viral B cells and T helper cells in this process will also be investigated. The team will try to discover which viral proteins and epitopes are involved in the protective immune response by constructing mutants of viral C strains. The new techniques developed during the project – the mutant C strain virus constructs, together with various reagents – will be used to gain insights into the properties of the immune responses. Finally, the partners will approach pharmaceutical companies to help commercialise any promising vaccine candidates and diagnostic assays emerging from this project.

### Contact

#### Tiny de Bruin

Animal Sciences Group, Wageningen  
UR Division Infectious Diseases  
Visiting address: Houtribweg 39  
P.O. Box 65 8200 AB Lelystad  
The Netherlands  
Tel: +31 320 238 684  
Fax: +31 320 238 668  
E-mail: [tiny.debruin@wur.nl](mailto:tiny.debruin@wur.nl)  
[www.asg.wur.nl](http://www.asg.wur.nl)



## Prevention of foot-and-mouth disease (FMD)

**FMD has the greatest economic impact of all viral diseases in farm animals. It is prevalent in many parts of the world, and there are numerous natural reservoirs of the virus. The lack of any general vaccination policy means that these latent sources of FMDV lie in wait to trigger new outbreaks at any time.**

Currently available vaccines can only afford protection against a limited number of variants of the virus (serotypes and sub-types). Similarly, they cannot prevent persistent infections setting in without the presence of symptoms – thereby creating carriers of the disease. For these reasons, in 2001 a Spanish-led consortium undertook the **FMDV vaccine** project: Biosafe coronavirus vector-based vaccine for prevention of foot-and-mouth disease. Its goal is the preparation of marked, safe and effective anti-FMD vaccines that will also prevent development of the carrier state in ruminants.

### Vaccine construction

The project team looked for a suitable vector from which to build the vaccine. It needed the following characteristics:

- to be marked to distinguish vaccinated from infected animals.
- able to prevent the emergence of the carrier state.
- able to offer adequate humoral and cellular immune responses.
- ideally, able to mimic the immune response to a natural infection.
- to be inexpensive and safe (in particular, free of infectious FMDV entities).
- be well tolerated, without serious side effects.

The partners found that vectors based on the transmissible gastroenteritis virus (TGEV) could meet these conditions. The main objective then was to construct a cDNA vector based on a TGEV system that expressed certain proteins of FMDV. The immune response to the vaccine that they built is different to the immune response to FMDV infection, as a result of the absence of specific FMDV antibodies, and to the specificity of a particular incorporated TGEV protein. There is no possibility of passing on the infectious virus

from these constructs because essential viral genes are missing.

### High hopes

Results after one year into the project were very promising with respect to the potency of the vaccine. The TGEV vectors were found to elicit strong immune responses (both humoral and cellular). It would also appear that the vaccine might be well tolerated since clinical symptoms following inoculation with it are milder than with an infection from TGEV itself. However, the instability of an inserted gene in the new constructs presents a major problem; some modifications have already been made and are being studied. Work being done to produce a suitable ELISA test should lead to a better assessment of the immune responses to these vector constructs.

Planned testing of the new vaccine in pigs will examine the immune responses, the systemic distribution of antigens and antibodies, and whether the virus is transmitted after infection with FMDV – i.e. to check for potential carriers of the disease.

### Contact

#### Esteban Domingo

Centro de "Biología Molecular Severo Ochoa"  
CSIC Cantoblanco

28049 Madrid

Spain

Tel: + 34 91 397 84 85

Fax: + 34 91 397 47 99

E-mail: edomingo@cbm.uam.es

## Improved DNA vaccines against FMDV

**DNA vaccines against foot-and-mouth disease virus (FMDV) have several advantages over the inactivated vaccines currently in use in the EU. Infectious viruses are not involved in their preparation, so the risk of contamination is reduced. They are also cheaper and, since they incorporate an incomplete virus genome, DNA vaccines permit the differentiation of vaccinated from infected animals.**

A **FMDna vaccine** project – Optimising DNA-based vaccination against FMDV in sheep and pigs – began in September 2002 and follows on from previous work on DNA vaccination that succeeded in partially protecting pigs against FMD. The research team counts four partners from three European countries. They are attempting to produce and evaluate several new DNA vaccines, together with a range of suitable adjuvants (e.g. alum and different kinds of charged particles). They are also trying to determine optimal vaccination protocols, especially for the route of administration. But first and foremost is the ability of these new DNA vaccines to induce protective immune responses in the animals at an early stage of infection.

### New tool kit

Some significant achievements were made during the first year of the project:

- **New virus-derived plasmids** were prepared for DNA vaccination, containing the FMDV 'empty capsid' gene.
- **12 new plasmids** expressing T- and B-cell epitopes have been prepared.
- **Specialised carrier molecules** were developed for FMDV epitopes. Immunisation of mice making use of these molecules is now underway.
- **Experiments** are in progress with cationic microspheres on which DNA vaccine is adsorbed – DNA vaccination in sheep has already begun. Several potential adjuvants are being evaluated. These experiments should yield considerable information about the immune responses elicited by DNA vaccines. In parallel, a dose-finding experiment was conducted in pigs comparing the effects of particular plasmids on immune responses. As expected, a higher dosage enhanced vaccine efficacy. Another

experiment is being carried out in pigs to determine the degree of early protection (at five days) induced by a single administration of *Pseudorabies* virus DNA vaccine.

During the remainder of the project, the partners will concentrate on comparing the immunogenicity of different FMDV antigens. Once the best DNA vaccines have been selected, immunisation protocols will be evaluated, especially 'prime/boost' regimens (using two different vectors, one to prime and the other to boost the induced immune responses). Another step is to see whether the vaccines can be delivered via the mucosae, especially through the nose.



### Contact

**Bernard Charley**

INRA

Département de santé animale  
Virologie et Immunologie moléculaires

78350 Jouy-en-Josas

France

Tel: +33 1 34 65 26 20

Fax: +33 1 34 65 26 21

E-mail: [charley@jouy.inra.fr](mailto:charley@jouy.inra.fr)

<http://www.inra.fr/sa>



## Eliminating the carrier state



**Vaccines were not part of the control strategies during recent devastating outbreaks of foot-and-mouth disease (FMD), mainly because currently available vaccines cannot distinguish carriers of the disease from vaccinated animals. The solution adopted in the EU has been the wide-ranging slaughter of herds. A new project could be the starting point for finding agents that either prevent infection or cure carriers of the disease.**

The **FMD Tropism** research project, Foot-and-mouth disease virus: the molecular basis of tissue tropism and persistence, makes use of the recent invention of laser micro-dissection (LMD). The LMD microscope allows minute sections, down to individual cells, to be cut from biological material. These can then be analysed to demonstrate changes in gene expression during an infection or shown by carriers of FMD virus. Tracking the changes in protein expression in the host cells allows the progression of disease to be followed at the molecular level.

### Tracking the virus

FMD virus accumulates in epithelial cells, especially in those located in the pharynx, and forms a focus of persistent infection there. Integrins (receptor proteins with specific cell adhesion function) have been identified as receptors for the virus. However, until now very little has been known about the mechanism of integrin-FMDV interaction in live animals. This project aims to examine expression levels of certain integrins in the upper respiratory tract of animals during infected states. It will attempt to discover how the virus selects these particular receptors.

The project partners will use LMD to investigate the expression of integrins in the upper respiratory tract of cattle and pigs and correlate the findings with sites of viral replication. They aim to determine whether or not the carrier state is maintained by adaptive

mutations in the virus. The other possibility is that integrin expression in the epithelial cells changes during infection to encourage persistent infection at a particular body site.

#### Contact

##### Thomas Wileman

Institute for Animal Health  
Pirbright Laboratory  
Ash road, Pirbright  
Woking, Surrey, GU24 0NF  
UK

Tel: +44 1482 232441

Fax: +44 1483 232448

E-mail: [thomas.wileman@bbsrc.ac.uk](mailto:thomas.wileman@bbsrc.ac.uk)



## Control of classical swine fever (CSF) in wild boar



**In many European countries, wild boar act as a large reservoir for classical swine fever virus (CSFV). As such, they present an ever-present risk of transmission of disease to farmed animals. To ensure the effectiveness of EU strategies for preventing the transmission of CSFV to domestic pigs, an effective vaccine is needed for use in wild boar. Its success would significantly boost the competitiveness of the European pork industry.**

There is plenty of epidemiological information on the spread of CSF infection in the wild and on the effects of immunisation and other control measures. This could be used to gain a better

understanding of the transmission of the virus, but a scientific evaluation of these data has never been done. This will be the first step for nine partners from seven European countries in the **CSF vaccine and wild boar** project: Epidemiology and control of classical swine fever (CSF) in wild boar and potential use of a newly developed live marker vaccine. They aim to build an epidemiological and economic model for CSF eradication in wild boar. The process will be assisted by data from a recently created European database for CSF monitoring by one of the partners. A cost-benefit analysis for the management of the infection in wild boar populations will complete the model and will be used to evaluate different control strategies – e.g. vaccination, culling, or no action at all.

### Creating a vaccine

The project partners will carry out studies in order to develop a live marker vaccine for the immunisation of wild boar against CSF. Modified live CSFV vaccines, such as the 'C-strain' vaccine, represent a gold standard for protection against CSF infection and are also suitable for oral administration. Live attenuated vaccines are known to induce a long-lasting immune response, but the danger is that the vaccine virus can replicate in an animal and be transmitted to other animals. Therefore, the project team will try to generate a live virus vaccine which has a modified genome that is host-specific and will not be transmitted. The genomic markers will allow the application of specific tests (like ELISA and RRT-PCR) which will discriminate between the vaccinated animals and the naturally infected ones.



When the new candidate marker vaccine eventually becomes available, it will be combined with baits to lure the boars to eat it. These baits will contain the live attenuated CSFV marker vaccine.

It has been shown that the genetic material in the CSFV genome may be quite extensively manipulated. Infectious cDNA clones of CSFV permit alterations such as insertion of foreign genes, exchange of genes between related strains, and the introduction of mutations with different functional properties. During the course of the project, a number of viruses with defined properties will be created in parallel. At the same time, the partners will develop appropriate diagnostic tests and, because a quick diagnosis of CSF is very important, this will include a pen-side test. These new diagnostic tools will enable discrimination between vaccinated and naturally infected animals.

## Better CSF control

Another part of the project is focusing on a better understanding of CSF transmission after vaccination, which is crucial to improve CSF control. Therefore, the organ distribution of the actual CSFV field strain circulating in wild boar, and the organ distribution of the developed live marker vaccine will be studied. Pigs infected but not sick – i.e. carriers – will also be studied for virus transmission since this may well be a major problem in eradicating CSF or in interpreting diagnostic tests.

### Contact

#### Frank Koenen

Department of Virology  
Modelisation of Epizootic Diseases Section  
CODA-CERVA  
Groeselenberg 99  
B-1180 Ukkel Belgium  
Tel: +32 2 379 05 18  
Fax: +32 2 379 06 70  
E-mail: [frank.koenen@var.fgov.be](mailto:frank.koenen@var.fgov.be)



## Improved control of foot-and-mouth disease (FMD)



**Policy on FMD control in the EU is planned in the near future to adopt emergency vaccination followed by screening for residual infection to control future outbreaks. Since this will occur before new vaccines become available, current vaccines will have to be relied upon.**

To be able to implement a policy of emergency vaccination, diagnostic tests that can discriminate between residual infection and vaccination are essential. It has been shown that the detection of antibodies in the non-structural proteins (NSP) of

the FMD virus is a sensitive and specific method that can do this. Consequently, tests for antibodies to the NSP component of the virus will be developed.

### Two-pronged approach

Ten participants, each from different European countries, have joined forces in the project **FMD-ImproCon** – Improvement of foot-and-mouth disease control by ethically acceptable methods based on scientifically validated assays and new knowledge on FMD vaccines, including the impact



of vaccination. The research hinges on two main objectives: the validation of NSP-based tests, and the improvement of existing tests.

Experiments will provide information on the impact of vaccination on the carrier state, virus dissemination, the onset of immunity, the potency of the vaccine in emergency use, vaccine strain selection, and new marker vaccines. The outcome will be the development of vaccines providing specific mucosal immunity, since this type of immunity can prevent the virus causing local infections and inducing the carrier state.

The ten research teams will cover six sets of tasks:

- **validation** of existing NSP-tests.
- **development** and validation of confirmatory and new NSP-tests (using ELISA methods).
- **improved FMDV detection.**
- **impact of vaccination** on virus dissemination and the carrier state.
- **improved vaccine** strain selection.
- **development** of a new marker vaccine.

#### Contact

##### Kris De Clercq

Department of Virology  
Epizootic Diseases Section  
CODA-CERVA-VAR  
Groeselenberg 99  
B-1180 Ukkel  
Belgium  
Tel: + 32 2 3790 512  
Fax: + 32 2 3790 666  
E-mail: kris.de.clercq@var.fgov.be



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The frequent outbreaks of transmissible diseases like foot and mouth disease (FMD), classical swine fever (CSF) and avian influenza (bird flu) have caused devastating economic losses in the past years. Organised by the European Commission, a workshop on EU funded research in FMD and CSF brought together researchers, policy makers, international organisations and stakeholders from all over the world. Relying on the results of this workshop, it is the intention of this publication to present ongoing research activities funded by the EU in the context of outbreaks of these epizootic diseases and the new EU legislation in this field. Future research needs and ways to boost international co-operation in research are also examined.

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